Quality Assurance Project Plan

Wolf Run Watershed Based Plan

EPA 319(h) Grant No. C9994861-09

Prepared By:
Third Rock Consultants, LLC
2526 Regency Road Suite 180, Lexington KY 40503
859-977-2000

Prepared For:
Lexington-Fayette Urban County Government
Department of Environmental Protection
200 East Main Street, Lexington, KY 40507
859-425-2800

and

Kentucky Department for Environmental Protection
Division of Water
200 Fair Oaks Lane
Frankfort, KY 40601
502-564-3410

Date: April 11, 2011

Revision Date: -

Revision No.: 0

This Page Intentionally Blank

SECTION A – PROJECT MANAGEMENT

A1. Title and Approval Sheet

Quality Assurance Project Plan
for Wolf Run Watershed Based Plan

Steve Evans / QAPP Author and Project Quality Assurance Officer, Third Rock Consultants, LLC	April 12, 2011 Date
Susan Bush / Project Manager, Lexington-Fayette Urban County Government	Date
David Price / Laboratory Manager, Lexington-Fayette Urban County Government	Date
Ken Cooke / Volunteer Coordinator, Friends of Wolf Run	Date
Brook Shireman / Project Manager, Kentucky Division of Water	Date
Lisa A. Hicks / Quality Assurance Officer, Kentucky Division of Water	Date

This Page Intentionally Blank

Revision History

This page documents the revisions over time to this document. The most recent iteration should be listed in the first space, with consecutive versions following. Signatures may be required for revised documents.

Date of Revision	Page(s)/Section(s) Revised	Revision Explanation
04/12/11	All	Original preparation date of document

A2. Table Of Contents

SECTION A – PROJECT MANAGEMENT 3
A1. Title and Approval Sheet 3
A2. Table Of Contents 6
A3. Distribution List 9
A4. Project / Task Organization 10
A5. Project Definition / Background 11
A6. Project/Task Description 13
A6.1. General Overview of Project 13
A6.1.1. Karst Hydrograph Characterization 17
A6.1.2. Conductivity Survey 17
A6.1.3. Benthic Macroinvertebrate Collection 17
A6.1.4. Watershed Habitat Assessments 18
A6.1.5. Hydrogeomorphic Assessment 18
A6.1.6. Water Quality Monitoring 19
A6.2. Project Timetable 19
A7. Data Quality Objectives (DQOs) and Criteria for Measurement Data 22
A7.1. Data Quality Objectives 22
A7.2. Data Quality Indicators 24
A7.2.1. Precision 24
A7.2.2. Accuracy 25
A7.2.3. Representativeness 26
A7.2.4. Completeness 26
A7.2.5. Comparability 27
A7.2.6. Sensitivity 27
A8. Training Requirements 27
A9. Documentation and Records 27
A9.1. Field Documentation and Records 27
A9.2. Laboratory Documentation and Records 28
A9.3. Quality Documentation and Final Reports 29

SECTION B. - DATA GENERATION AND ACQUISITION 30
B1. Sampling Process Design 30
B1.1. Sampling Site and Reach Locations 30
B1.2. Sampling Design Rationale 32
B1.2.1. Karst Hydrograph Characterization 32
B1.2.2. Conductivity Survey 34
B1.2.3. Benthic Macroinvertebrate Collection 34
B1.2.4. Watershed Habitat Assessments 34
B1.2.5. Hydrogeomorphic Assessment 34
B1.2.6. Water Quality Monitoring 35
B2. Sampling Methods 36
B2.1. Sampling Equipment 36
B2.2. Sampling Methods 36
B2.2.1. Karst Hydrograph Characterization 36
B2.2.2. Conductivity Survey 39

B2.2.3. Benthic Macroinvertebrate Collection 40
B2.2.4. Watershed Habitat Assessments 44
B2.2.5. Hydrogeomorphic Assessment 48
B2.2.6. Water Quality Monitoring 50
B3. Sample Handling and Custody Requirements 53
B3.1. Chain-of-Custody 53
B3.2. Sample Handling and Transport 54
B3.3. Sample Labeling 54
B3.4. Sample Designation 55
B4. Analytical Methods Requirements 55
B5. Quality Control Requirements 56
B5.1. Field Quality Controls 56
B5.2. Macroinvertebrate Taxonomic Quality Controls 56
B5.3. Chemical Laboratory Quality Controls 57
B6. Instrument / Equipment Testing, Inspection, Calibration, and Maintenance Requirements 57
B7. Data Management 58

SECTION C – ASSESSMENT AND OVERSIGHT 59

SECTION D – DATA VALIDATION AND USABILITY 61
D1. Data Review, Validation and Verification 61
D2. Validation and Verification Methods 62
D3. Reconciliation with User Requirements and Data Quality Objectives 63

SECTION E. - REFERENCES AND CITATIONS 63

List of Exhibits

Exhibit 1 – Project Sampling Sites 14
Exhibit 2 – Karst System Hydraulic Monitoring 15
Exhibit 3 – Watershed Habitat Assessment Streams 33

List of Tables

Table 1 – Project Roles and Responsibilities 11
Table 2 – Monitoring Activity Overview 13
Table 3 – Sampling Equipment Summary 16
Table 4 – Project Schedule 21
Table 5 – Summary of Data Quality Objectives and Standard Operating Procedures 23
Table 6 – Acceptance Criteria for Water Chemistry and *In-Situ* Measurements 24
Table 7 – Completeness Goals of Sampling Activities 26
Table 8 – Sampling Locations 31
Table 9 – Habitat Assessment Stream Segments 32
Table 10 – Sample Preservation and Hold Time 36
Table 11 – Summary of Sampling Methods for Macroinvertebrates 41
Table 12 – Field Equipment Calibration and Maintenance 57

Table 13 – Watershed Assessment and Management Reports 60
Table 14 – Data Qualifiers and Response..... 62

List of Figures

Figure 1 – Wolf Run Watershed Monitoring Organization Chart 10
Figure 2 – Measurement of Discharge Through Sub-Sectional measurements..... 37

List Of Appendices

Appendix A – Datasheets
Appendix B – Standard Operating Procedures

A3. Distribution List

The following individuals will receive the approved Quality Assurance Project Plan (QAPP) and any subsequent revisions.

Name	Address	Phone Number	E-mail Address
Steve Evans Third Rock Consultants, LLC	2526 Regency Road, Suite 180 Lexington, KY 40503	859-977-2000	sevans@thirdrockconsultants.com
Gerry Fister Third Rock Consultants, LLC	2526 Regency Road, Suite 180 Lexington, KY 40503	859-977-2000	gfister@thirdrockconsultants.com
Marcia Wooton Third Rock Consultants, LLC	2526 Regency Road, Suite 180 Lexington, KY 40503	859-977-2000	mwooton@thirdrockconsultants.com
Bert Remley Third Rock Consultants, LLC	2526 Regency Road, Suite 180 Lexington, KY 40503	859-977-2000	bremley@thirdrockconsultants.com
Jennifer Shelby Third Rock Consultants, LLC	2526 Regency Road, Suite 180 Lexington, KY 40503	859-977-2000	jshelby@thirdrockconsultants.com
Susan Bush Lexington-Fayette Urban County Government	200 East Main Street, Lexington, KY 40507	859-425-2800	sbush@lfucg.com
David Price Lexington-Fayette Urban County Government	301 Lisle Industrial Avenue Lexington, Kentucky 40511	859-425-2415	dprice@lfucg.com
Jason Backus Kentucky Geological Survey	228 Mining & Mineral Resources Building University of Kentucky Lexington, KY 40506-0107	859-323-0555	jbackus@uky.edu
Ken Cooke Friends of Wolf Run	639 Cardinal Lane Lexington, Kentucky 40503	859-940-8234	ken.cooke@kbcnet1.com
Brooke Shireman KY Division of Water	200 Fair Oaks Lane Frankfort, KY 40601	502-564-3410	brooke.shireman@ky.gov
Lisa A. Hicks, KY Division of Water	200 Fair Oaks Lane Frankfort, KY 40601	502-564-3410	lisa.hicks@ky.gov

A4. Project / Task Organization

This Quality Assurance Project Plan (QAPP), prepared by Third Rock Consultants, LLC (Third Rock), is to be reviewed and approved by the Kentucky Division of Water (KDOW) and the Lexington-Fayette Urban County Government (LFUCG). This QAPP establishes the planning, implementation, and assessment procedures necessary to meet the minimum data quality objectives (DQOs) for the monitoring of the Wolf Run watershed.

Third Rock is committed to producing quality data that will assist the KDOW and LFUCG in obtaining the information necessary to facilitate the development of a Wolf Run Watershed Based Plan. This QAPP is designed to provide a complete plan for achieving all project data quality objectives. However, effective communication is essential to ensure that all parties properly implement the plan. Any project related quality feedback, questions, or concerns should be communicated to the project administrator or quality manager to facilitate appropriate analysis and resolution.

The implementation of the monitoring plan requires the effective operation of the project team. Figure A-1, Wolf Run Watershed Monitoring Organizational Chart, identifies the parties that comprise the Project Team and the lines of authority and communication under which this team operates. The specific roles and responsibilities of each key party are documented in Table 1.

FIGURE 1 – WOLF RUN WATERSHED MONITORING ORGANIZATION CHART

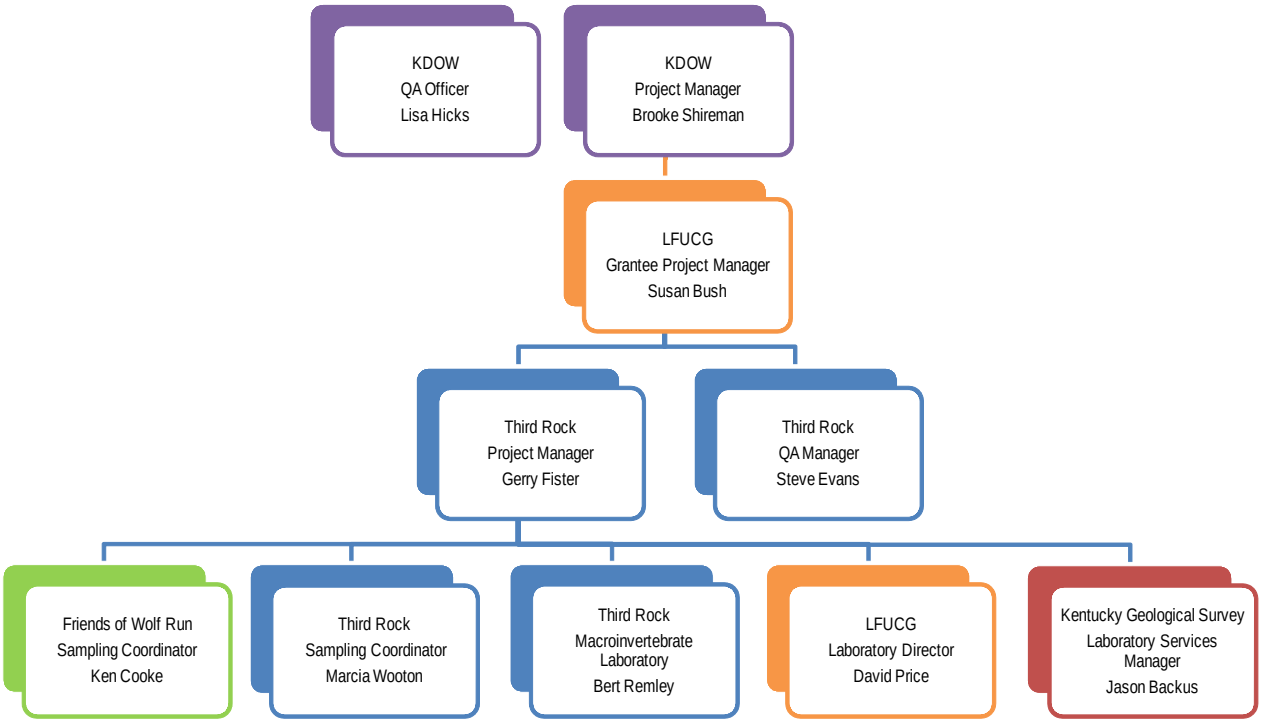


TABLE 1 – PROJECT ROLES AND RESPONSIBILITIES

Name	Project Title	Responsibility
Brooke Shireman KDOW	KDOW Project Manager	Administratively oversees data collection planning and reviews data
Lisa Hicks KDOW	KDOW QA Officer	Administratively oversees QAPP conditions; may assist in review of data
Susan Bush LFUCG	Grantee Project Manager	Oversight of project
Gerry Fister Third Rock	Project Manager	Project scheduling and coordination to meet time line and budget goals
Steve Evans Third Rock	QA Manager	Development of the QAPP; ensure QAPP compliance, conduct audits, review and approve all data generated; preparation of QA reports
Marcia Wooton Third Rock	Sampling Coordinator	Coordination of water quality sample collection events with volunteer staff; bottle preparation and labeling, laboratory communication on hold times and data results
Ken Cooke Friends of Wolf Run	Volunteer Sampling Coordinator	Coordination of volunteer sampling activities; training volunteers per QAPP specifications
David Price LFUCG	Laboratory Director	Chemistry laboratory analysis for water quality samples
Jason Backus Kentucky Geological Survey	Laboratory Services Manager	Chemistry laboratory analysis for water quality samples
Bert Remley Third Rock	Macroinvertebrate Laboratory Chief Taxonomist	Oversee and conduct field biological sampling; biological data review; identification of benthic samples; oversee macroinvertebrate quality assurance

A5. Project Definition / Background

Wolf Run was first listed as impaired for swimming use (non-support) in the 1998 303(d) list of Kentucky impaired waters. This impaired status has remained since that time with additional impairments (partial support of warmwater aquatic habitat use and non-support of secondary contact use) being identified in subsequent years (KDOW 2010a). The impairment of Wolf Run, in addition to other Lexington streams, led the US Environmental Protection Agency (USEPA) and the Kentucky Environmental and Public Protection Cabinet (KY EPPC) to file a lawsuit against Lexington in 2006 for violations of the Clean Water Act in 2006. The lawsuit was due to failure of the city to maintain the sanitary and storm sewer systems, which caused raw sewage discharges into streams. On March 14, 2008 LFUCG entered into a Consent Decree in order to resolve this lawsuit (United States, 2006). Within the Consent Decree, LFUCG agreed to make extensive improvements to its sewer systems and address sanitary sewer overflows and associated MS4 permit violations, as well as to reduce the discharge of pollutants via stormwater. With the Consent Decree in place, LFUCG is furthering its efforts to improve water quality in Wolf Run.

The citizens of Lexington, especially those in the Wolf Run watershed, share the interest in water quality improvement with LFUCG. The Friends of Wolf Run (FOWR), a community based watershed group, became active in the watershed in 1997, prior to the first impaired listing of Wolf Run, educating the community about stream health and making initial steps toward a cleaner watershed. This group continues to be an outspoken proponent of improving the water quality in Wolf Run. The FOWR sponsors the Wolf Run Watershed Council, consisting of groups and individuals working to improve the watershed.

A watershed plan is being developed in order to provide a comprehensive assessment of the health of the watershed, citizen and stakeholder concerns, watershed remediation strategies, and implementation plans for the future. This is being developed under a Section 319(h) Nonpoint Source Implementation Program Cooperative Agreement (#C9994861-09) awarded by the Commonwealth of Kentucky, Energy and Environment Cabinet, Department for Environmental Protection, Division of Water (KDOW) to LFUCG based on an approved work plan. These federal funds were awarded to KDOW by the EPA under Section 319(h) of the Clean Water Act. Third Rock was selected as the environmental consultant for work under this grant through a request for proposal issued by LFUCG. FOWR was also issued grant funding through a memorandum of agreement with LFUCG, primarily to engage, educate, and solicit input from the public during the development of this plan.

In the development of the Wolf Run Watershed Based Plan (WBP), all known and relevant existing information pertaining to the watershed was compiled and evaluated for data quality. The purpose of the data compilation and assessment was to thoroughly describe the Wolf Run watershed and to determine what additional data would be necessary in order to identify the impairments in the watershed and their causes and sources, to calculate the extent of the impairments, and to determine solutions for improving water quality. Based on this analysis, six major sampling needs were identified, which include:

- measurements to characterize of the discharge hydrograph for the Preston Springs karst basin
- watershed conductivity survey
- macroinvertebrate collections on tributaries and headwaters
- watershed-wide habitat assessments
- hydrogeomorphic assessment of the watershed
- a water quality monitoring data set meeting the specifications of KDOW's "Watershed Planning Guidebook for Kentucky Communities" (KWA and KDOW, 2010)

This QAPP will establish the quality criteria and collection process necessary to produce data which will fill the identified gaps and allow for the determination of the locations in the watershed in which BMPs will be most feasible, efficient, and effective.

A6. Project/Task Description

A6.1. General Overview of Project

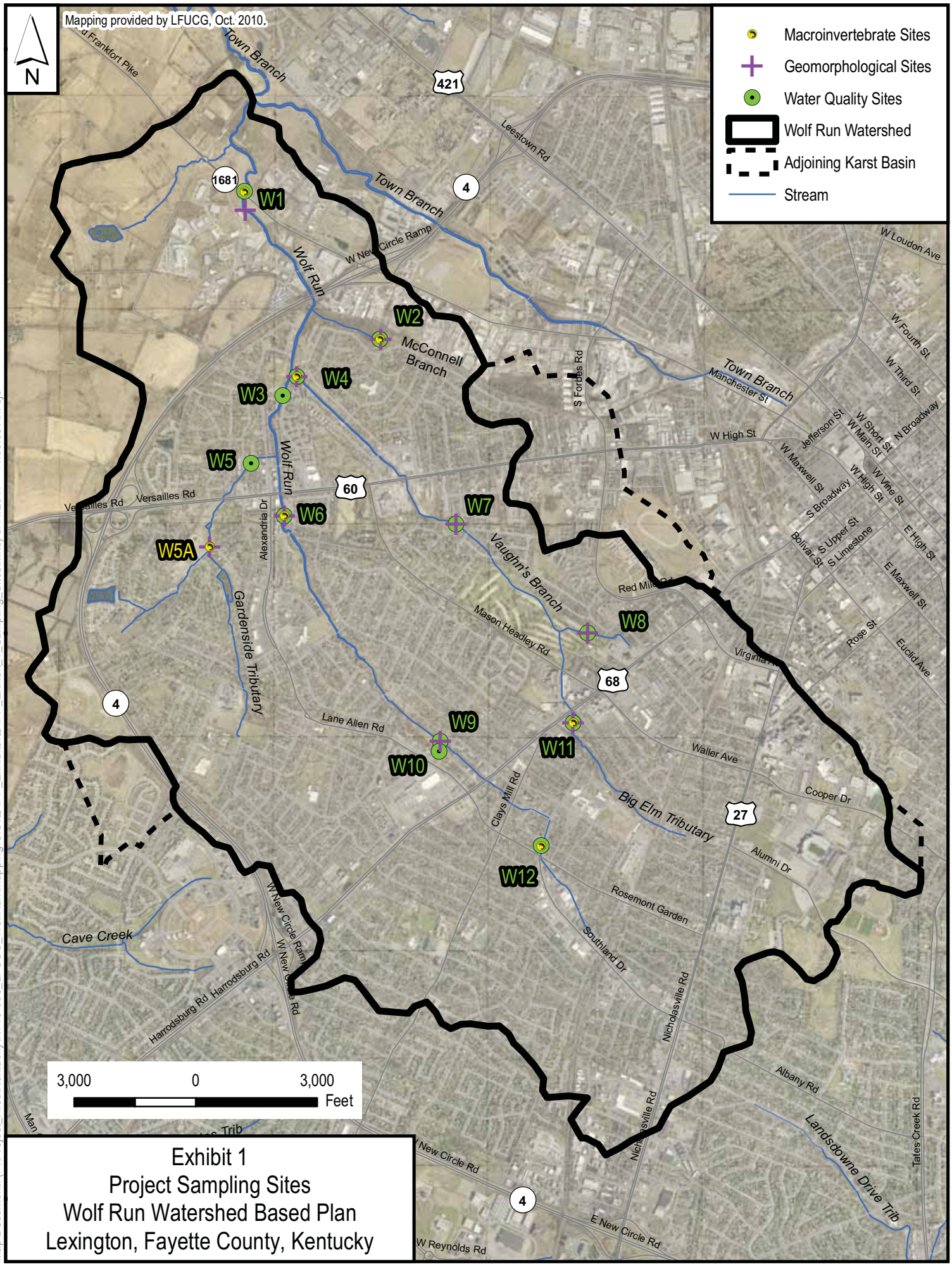
This project will involve six different monitoring activities, as follows:

- karst hydrograph characterization
- conductivity survey
- benthic macroinvertebrate collection
- watershed habitat assessment
- hydrogeomorphic assessment
- water quality monitoring

The sampling responsibilities, frequencies and number of sites to be sampled for each monitoring activity are summarized in Table 2. The equipment necessary for each of these activities is specified in Table 3. Exhibits 1 and 2 show the study area and the selected sampling locations. A discussion of each of these activities follows.

TABLE 2 – MONITORING ACTIVITY OVERVIEW

Monitoring Activity	Sampling Responsibility	Frequency	No. of Sites
Karst hydrograph characterization	Third Rock	Twice	6
Conductivity survey	Volunteers	Once	1/ 100 ft
Benthic Macroinvertebrate Collection			
a. Macroinvertebrate Collection	Third Rock	Once	6
b. Habitat Assessment	Third Rock and Volunteers	Once	6
Watershed Habitat Assessments	Volunteers	Once	At least 1 per Segment (24)
Hydrogeomorphic Assessment	Third Rock	Twice	9
Water Quality Monitoring			
a. Water Quality Monitoring	Third Rock and Volunteers	Monthly, 10 events	12
b. *E. coli* Geomean Sampling	Third Rock and Volunteers	5 events in 30 days	12


Mapping provided by LFUCG, Oct. 2010.
N
Macroinvertebrate Sites
Geomorphological Sites
Water Quality Sites
Wolf Run Watershed
Adjoining Karst Basin
Stream
W1
W2
W3
W4
W5
W5A
W6
W7
W8
W9
W10
W11
W12
Town Branch
Wolf Run
McConnell Branch
Vaughn's Branch
Gardenside Tributary
Big Elm Tributary
Cave Creek
Lane Allen Rd
Mason Headley Rd
Red Mile Rd
Versailles Rd
Harrodsburg Rd
Leestown Rd
W New Circle Rd
E New Circle Rd
W Reynolds Rd
Albany Rd
Landsdowne Drive Trib
Tates Creek Rd
Nicholasville Rd
Clays Mill Rd
Southland Dr
Rosemont Garden
Alumni Dr
Cooper Dr
Waller Ave
Euclid Ave
Rose St
E Maxwell St
E High St
W Maxwell St
W High St
W Vine St
W Short St
W Main St
W Fourth St
W Third St
W Loudon Ave
N Broadway
S Broadway
S Upper St
S Limestone
Bolivar St
Jefferson St
Manchester St
W High St
S Forbes Rd
Alexandria Dr
421
1681
4
60
68
27
3,000
0
3,000
Feet
Exhibit 1
Project Sampling Sites
Wolf Run Watershed Based Plan
Lexington, Fayette County, Kentucky
Map Document: (P:\Project_Files\Kentucky\KY10-030_LFUCG_WolfRunMapping\GIS\QAPP_Exhibits\QAPP_Exhibit_1_Sampling_Sites.mxd) 3/11/2011 -- 11:00:05 AM sje

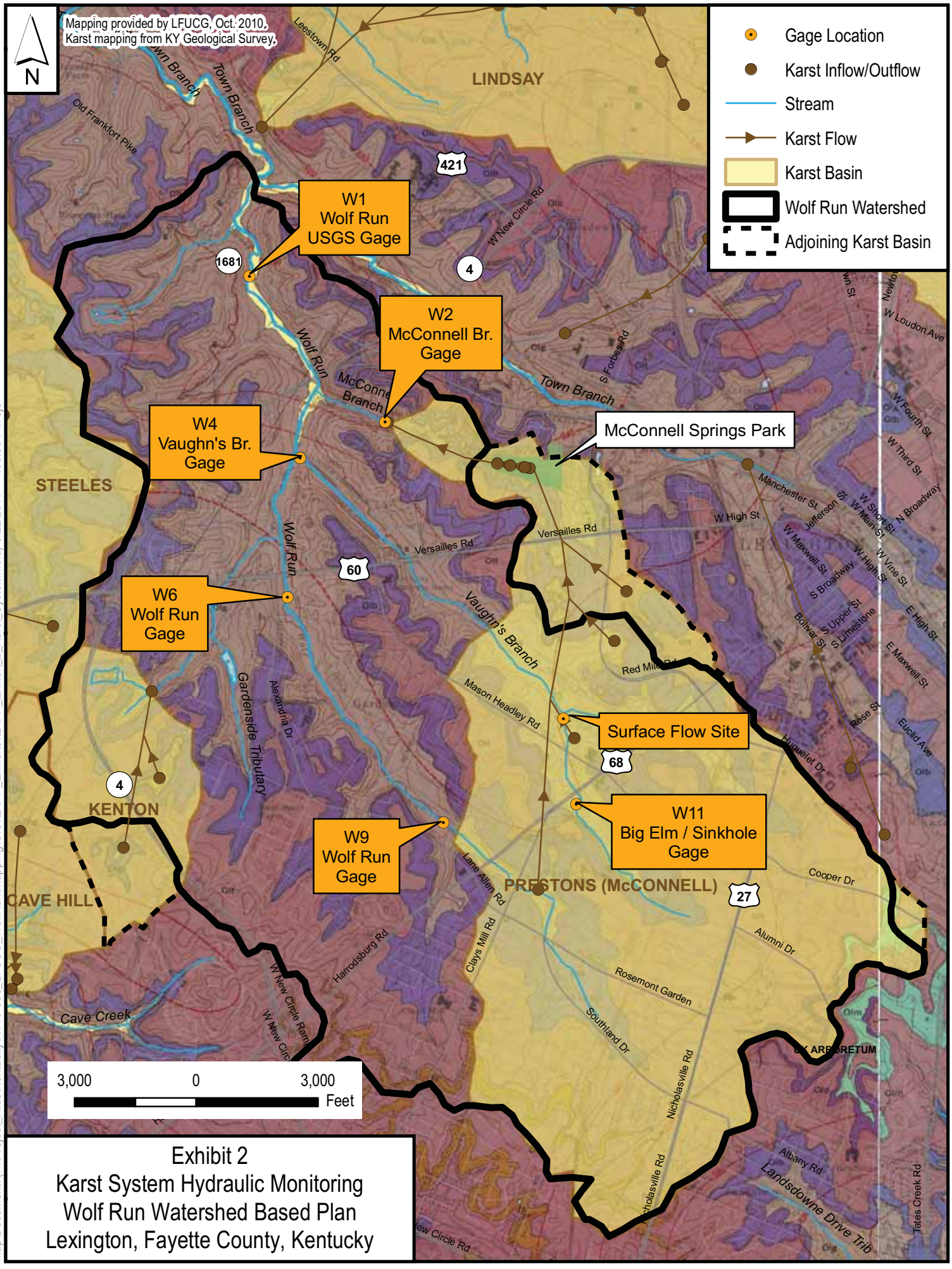


Exhibit 2
Karst System Hydraulic Monitoring
Wolf Run Watershed Based Plan
Lexington, Fayette County, Kentucky

Map Document: (P:\Project_Files\Kentucky\KY10-030_LFUCG_WolfRunMapping\GIS\QAPP_Exhibits\QAPP_Exhibit_2_Karst_System.mxd) 3/22/2011 -- 4:30:55 PM sje

TABLE 3 – SAMPLING EQUIPMENT SUMMARY

Monitoring Activity	Equipment	
Karst hydrograph characterization	Marsh McBirney Flo-Mate Portable Flowmeter Stopwatch Tape measure (100 feet in 1/10ft increments) Top-setting wading rod Five gallon bucket Field Notebook	5 Level TROLL 1 Baro TROLL RuggedReader and communication cables PVC pipe, elbow, and caps Steel cable Lap top computer Pliers Wrench Hammer Metal fasteners Steel fence post
Conductivity survey	Digital Camera Field Datasheet	EC Conductivity PockeTesters, or equivalent
Benthic Macroinvertebrate Collection	GPS 600µm mesh, 0.25 meter wide rectangular net or kick seine 800 x 900µm D-frame dip net U.S. Number 10 sieve U.S. Number 30 sieve 2- 600µm mesh wash buckets	Medium-sized bucket 300µm nitex sampler/mesh Fine-tipped forceps 95% ethyl alcohol White picking pans Sample jars
Habitat Assessment	Digital Camera High-Gradient Habitat Assessment Field Data Sheet	GPS Site Characterization Form
Hydrogeomorphic Assessment	Gravelometer GPS Laser level & tripod 100ft & 50ft tapes RiverMorph on Rugged Reader	0.75-inch rebar Hammer Tape measure Surveying rod Field notebook
Water Quality Monitoring and *E. coli* Geomean Sampling	*Field Measurements* EC Conductivity PockeTesters Dissolved Oxygen Water Quality Test Kit (LaMotte Code 7414) 2 pH Wide Range Indicator Kits (LaMotte P-5085 and P-5100) Armored Thermometers (-5° to 45°C in 0.5°C increments) *All Sampling* Sample coolers Ice Plastic food storage bags Sample jars and preservatives Powderless latex or nitrile gloves Chain-of-custody Permanent marker Clear masking tape Blue or black ink pen	*Flow measurement* Marsh McBirney Flo-Mate Portable Flowmeter, or equivalent Stopwatch Tape measure (100 feet in 1/10ft increments) Top-setting wading rod Five gallon bucket Field Notebook *Field Filtration* 47mm magnetic filter funnel 1L Nalgene flask Teflon or Tygon tubing Forceps 0.45µm sterile membrane filters Deionized water

A6.1.1. Karst Hydrograph Characterization

To determine the influence of the karst system on the discharge and the nature of the stream hydrograph, simultaneous gaging of the three affected tributaries and a major sinkhole will be performed during base flow conditions and during a wet weather event. Temporary water level gages (pressure transducers with data loggers) will be installed at each of the five gaging stations.

Flow measurements will be conducted according to KDOW's *Measuring Stream Discharge Standard Operating Procedure* (KDOW 2010b). A minimum of 5 percent replicate measurements will be made during this gaging effort. The base flow event will consist of a single flow measurement at each of six gaging stations as shown on the attached Exhibit 2. It is anticipated that the base flow period will begin in mid-summer and extend to early fall. The wet weather event will target a storm event that is expected to have uniform rainfall across the watershed with expected accumulation of over 1 inch. The gaging will be performed by two teams of surveyors circulating to each of the five gaging points a minimum of every thirty minutes during the storm event. Monitoring will continue until well past the hydrograph peak.

A6.1.2. Conductivity Survey

During medium to low-flow conditions (0.5 to 5 cfs at the USGS gage), the Wolf Run watershed will be surveyed by *in situ* field temperature and specific conductance measurements to identify locations of "jumps" in the specific conductance levels as possible locations of pollution. Using GPS data loggers, field meters, data sheets, and photographs, all streams and tributaries (approximately 13.5 miles) will be measured at approximately 100-foot intervals (approximately 700 locations). Volunteer samplers will be trained to perform this study. Unless interrupted by a precipitation event, the survey should be completed over a one-week period. If a precipitation event occurs during this period, the survey will resume when water levels are consistent with the initial survey conditions.

A6.1.3. Benthic Macroinvertebrate Collection

Macroinvertebrate samples will be collected at six sites within the Wolf Run watershed in addition to the site at the mouth of Wolf Run, which is monitored for macroinvertebrates under the MS4 permit. The six sites are located on Vaughn's Branch, Big Elm Tributary, Cardinal Run, McConnell Branch, and two sites on Wolf Run (one upstream of Harrodsburg Road, one upstream of Versailles Road). These sites are identified on Exhibit 1.

The macroinvertebrate community at each site will be sampled using the recommended methods developed by KDOW (2009b, 2009c), which involve the collection of two separate samples, riffle and multihabitat. The riffle sample consists of four 0.25 meters^2 (m^2) samples collected from two separate riffles at each station using a 0.25 m^2 grid and a kicknet (600µm mesh). Riffle collections at each station will be composited to form one semi-quantitative sample. The qualitative, multihabitat sample includes, where habitat is available, samples from leaf packs; sticks/wood; bedrock/slabrock; undercut banks/submerged roots; aquatic macrophyte beds; soft sediment (using a U.S. # 10 sieve);

hand-picking of rocks (large cobble/small boulder) from riffles, runs, and pools; *aufwuchs* material off rocks, sticks, leaves, and filamentous algae; and visual searches of large woody debris. All samples collected with the dip net and the rock and wood samples will be processed through a 600µm wash bucket. Results of qualitative sampling from each microhabitat will be combined to form one composite sample for each station. Samples will be preserved in 95 percent ethanol and returned to the laboratory for processing and identification. All organisms will be identified to the lowest possible taxonomic level and recorded on laboratory data sheets. Random 300-specimen subsamples will be removed from the riffle samples using methods described by KDOW (2009b).

Habitat assessments will be performed by trained volunteers accompanied by Third Rock personnel at each of these sites. Visual assessments will be made to document riffle and pool substrates, stream channelization, riparian conditions and in-stream cover. Habitat assessment procedures will follow those outlined in *Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers* (Barbour et al. 1999).

A6.1.4. Watershed Habitat Assessments

In addition to the habitat assessments conducted at the macroinvertebrate sites, habitat assessments will be performed by trained volunteers throughout the watershed on parcel sized or 100m stream reaches. Using the visual-based habitat assessment procedures in Barbour *et al.* 1999, volunteers will survey as many segments as time permits within the 24 stream segments into which the watershed has been subdivided. At least one assessment will occur in each segment.

Information obtained from the habitat assessment will be used to supplement biological and physicochemical data when determining the overall health of the stream reach and stream-use designation. Additionally, habitat assessments will serve as a baseline to document physical changes that occur over time and to identify potential areas for BMP implementation.

A6.1.5. Hydrogeomorphic Assessment

Nine hydrogeomorphic monitoring sites have been preliminarily designated to measure channel changes in representative reaches. Assessment will include a series of spatially integrated, high-resolution cross-section and longitudinal profile surveys and streambed substrate evaluation to determine the extent of the effects of hydromodification in the Wolf Run watershed. Effects of hydromodification that may be revealed by the assessment include degree of bed and bank erosion, sedimentation, and habitat loss. The relative potential for improvement will also be qualitatively assessed based on the lack of obvious physical constraints in a reach, position in the landscape, or position in the watershed.

The baseline cross-section, profile and bed substrate will be compared to a subsequent survey to determine the degree and type of changes in physical structure and stream function that has occurred. Information obtained from the hydrogeomorphic assessment will be used to supplement biological, physicochemical, and habitat data when

determining the overall health of the stream reach and stream-use designation. This will also allow for the development of an understanding of the nature and location of the problems associated with channel modification. Hydrogeomorphic assessments will quantify physical stream changes that occur over time, help identify potential BMPs/implementation solutions, and prioritize reaches for implementation of those solutions.

A6.1.6. Water Quality Monitoring

Water quality monitoring will be conducted at ten (10) monthly sampling events at a minimum of twelve (12) sampling stations in the watershed during dry and wet conditions. The sampling date within each month will be flexible such that at least two of the events are considered 'wet-weather' and two of the events are considered 'dry-weather.' Sampling parameters will include discharge, *E. coli*, fecal coliform, total suspended solids, total phosphorus, ortho-phosphorus, ammonia, total kjeldahl nitrogen, nitrate, nitrite, total dissolved solids, turbidity, dissolved oxygen, specific conductance, temperature, and pH. The LFUCG Town Branch laboratory will analyze samples for *E. coli*, fecal coliform, total suspended solids, ammonia, nitrite, total dissolved solids, alkalinity, and hardness. The Kentucky Geological Survey will analyze samples for total phosphorus, ortho-phosphorus, total kjeldahl nitrogen, and nitrate. FOWR volunteer samplers will perform field measurements of turbidity, dissolved oxygen, specific conductance, temperature, and pH. Third Rock will accompany the volunteers during each event to conduct discharge monitoring and will field filter ortho-phosphorus samples. Also due to the short time period in which wet-weather events can be collected on the hydrographic rise, two wet-weather sampling events will be collected solely by Third Rock staff. If for some reason volunteers are not able to perform the sampling, Third Rock will collect all sampling parameters.

In addition to the monthly sampling, volunteers will collect an additional four events within a 30-day period during the Primary Contact Recreation period (May 1 to October 31) for *E. coli* and fecal coliform to evaluate the geometric mean for the primary contact period. A Third Rock staff member will accompany the volunteers during each event to conduct discharge monitoring. Only flow, *E. coli* and fecal coliform will be collected during these events. The LFUCG Town Branch laboratory will analyze the samples.

A6.2. Project Timetable

The project schedule for each of the monitoring activities as well as the data analysis, report completion, and watershed based plan section revisions are shown in Table 4.

The QAPP completion and approval is expected to occur in April 2011, prior to the initial volunteer training. If the QAPP approval is delayed beyond April, the monitoring schedule will be delayed a similar period. The first volunteer training event is scheduled for April 16, 2011, with four total training sessions planned. FOWR and its Science Advisors are to conduct these training sessions in accordance with this QAPP.

The Wolf Run Watershed Council will meet on a quarterly basis, at minimum. Progress reports on the monitoring activities will be presented at these meetings.

For the karst hydrograph characterization, data loggers will be installed in May 2011. Subsequent to their installation, the base flow event and wet weather event will be monitored during the respective weather conditions. A rainfall event of 1 inch will qualify as a wet weather event. Both events are expected to occur by the end of August 2011 if weather conditions allow. Data analysis will follow the events with the expected completion data of the report in May 2012.

Trained volunteer samplers will conduct the conductivity survey during medium to low-flow conditions (0.5 to 5 cfs at the USGS gage). Unless interrupted by a precipitation event, the survey will be completed over a one-week period such that the results reflect a "snapshot" of watershed conditions. If a precipitation event occurs during this period, the survey will resume when water levels are consistent with the initial survey conditions. The sampling will be performed during a period between May and September 2011. All hardcopy and electronic data will be submitted to Third Rock for data entry and analysis, with a final report expected by December 31, 2011.

Third Rock will sample macroinvertebrates in May 2011 during the appropriate sampling index period. For wadeable streams with a watershed >5 mi^2, the index period is May 1 to September 30; for headwater streams with a watershed <5 mi^2, it is February 15 to May 31. All sites are headwater sites except W1, which is sampled for macroinvertebrates under the MS4 permit and not under this project. Sampling will not occur during periods of excessively high or low flow or within two weeks of a known scouring flow event. Habitat assessments will be performed by trained volunteers concurrent with or within one week of the macroinvertebrate collections at each of these sites. Laboratory identification, metric calculation, data analysis, and report completion will occur prior to December 31, 2011.

The watershed habitat assessments performed within the 24 stream segments in the Wolf Run watershed will be performed subsequent to volunteer training and prior to October 31. All data will be compiled into an electronic database by the volunteer sampling coordinator and submitted to Third Rock by October 31, 2011.

Hydrogeomorphic monitoring will initially be conducted in May 2011 with a second survey to assess geomorphic conditions nine (9) months after the baseline is completed (February 2012), assuming sufficient flow events occur during this period. Data analysis will occur subsequent to the data generation, with a final report completed by April 30, 2012.

TABLE 4 – PROJECT SCHEDULE

Activity	2011									2012											
	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Planning and Training																					
QAPP Approval																					
Volunteer Training																					
Council Meetings																					
Quarterly	2nd	3rd		4th			5th			6th			7th			8th			9th		
Karst Hydrograph Characterization																					
Installation of Data loggers																					
Base Flow Event																					
Wet Weather Event		Rainfall dependent																			
Data Analysis																					
Report Completion																					
Conductivity Survey																					
Conduct survey		Complete within one week																			
Data Entry and Analysis																					
Report Completion																					
Benthic Macroinvertebrate Collection																					
Macroinvertebrate Collection	*Headwater Index*																				
Habitat Assessment	*Macro sites*																				
Laboratory identification																					
Data Entry and Analysis																					
Report Completion																					
Watershed Habitat Assessment																					
Habitat Assessments by Reach																					
Data Entry and Analysis																					
Report Completion																					
Hydrogeomorphic Assessments																					
Cross-sections, profiles, and pebble counts		Initial									Final										
Data Analysis																					
Report Completion																					
Water Quality Monitoring																					
Monthly Sampling (10)		1	2	3	4	5	6	7	8	9	10										
E. coli Geomean Sampling		4X in 30 days																			
Data Entry and Review																					
Loading Calculations and Source Determinations																					
Report Completion																					
Watershed Based Plan																					
Chapter 3: Monitoring																					
Chapter 4: Analysis																					
Chapter 5: BMPs																					
Chapter 6: Strategy																					
Chapter 7: Implementation																					

Water quality monitoring will be conducted monthly beginning in May and continuing until February 2012. At least two of the events are to be considered 'wet-weather' and two of the events are considered 'dry-weather.' Representative dry weather sampling conditions are defined by an antecedent period of 5 days during which there is no rainfall. Representative wet weather sampling conditions are defined by a period of at least 72 hours of dry weather preceding a rain event with a total accumulation of at least 0.1 inch. Sampling during 'wet-weather' will occur on the hydrographic rise. Additional sampling

for *E. coli* will occur four times within 30 days and is expected to begin in May, but may occur at any time within the Primary Contact Recreation period (May 1 to October 31). Third Rock will accompany volunteers during each event to conduct discharge monitoring. A minimum notice of 48 hours will be provided to FOWR prior to scheduling the volunteer sampling. The LFUCG Town Branch and Kentucky Geological Survey laboratories will analyze collected samples. Collection events shall be scheduled to avoid collection on Thursday, as this day is particularly busy for the Town Branch Laboratory. Expected turn-around-time for the laboratory analysis is 30 days. Chemical laboratory reports with data quality review by the Project QA Manager will be submitted to the project team within 60 days of sample delivery to the laboratory. With each sampling event, quality control samples including field duplicate samples will be collected along with the regular field samples.

The results of these studies will be used to complete the Wolf Run Watershed Based Plan according to the KDOW's "Watershed Planning Guidebook for Kentucky Communities" (2010) and the USEPA's *Handbook for Developing Watershed Plans to Restore and Protect Our Waters* (2008). The project schedule in Table 4 corresponds to the watershed plan chapters outlined in the KDOW guidance. The final plan, incorporating comments and recommendations, is scheduled for completion by December 31, 2012.

A7. Data Quality Objectives (DQOs) and Criteria for Measurement Data

A7.1. Data Quality Objectives

Data quality objectives (DQOs) are qualitative and quantitative statements that clarify the intended use of the data, define the type of data needed to support the decision, identify the conditions under which the data should be collected, and specify tolerable limits on the probability of making a decision error due to uncertainty in the data. The data quality objectives for the respective sampling activities are listed in Table 5, along with the Standard Operating Procedures associated with each of these activities. The overall objective of this project is to collect data of sufficient quality and quantity to support the development of a watershed-based plan for the Wolf Run watershed.

TABLE 5 – SUMMARY OF DATA QUALITY OBJECTIVES AND STANDARD OPERATING PROCEDURES

Sampling Activity	Objective	Standard Operating Procedures / References
Karst Hydrograph Characterization	Characterize the flow distribution between the surface water and ground water systems.	KDOW. 2010b. *Measuring Stream Discharge Standard Operating Procedure*. Kentucky Department for Environmental Protection, Division of Water, Frankfort, Kentucky. DOWSOP03019 In-Situ Inc. 2006. *Level TROLL® Operator's Manual*. www.in-situ.com
Conductivity Survey	Identify significant changes in conductivity levels to pinpoint sources of pollution	Oakton Instruments. Waterproof TDSTestr and ECTestr Series Instructions. http://www.4oakton.com/Manuals/ConductivityTDS/TDS_ECTestrmnl.pdf
Macroinvertebrate Collection and Identification	Calculation of the Macroinvertebrate Bioassessment Index (MBI). Macroinvertebrates have varying tolerances for water pollution and therefore can serve as long-term indicators of water quality	KDOW. 2009b. *Laboratory Procedures for Macroinvertebrate Processing and Taxonomic Identification and Reporting.* Kentucky Department of Environmental Protection, Division of Water, Frankfort, Kentucky. KDOW. 2009c. *Methods for Sampling Benthic Macroinvertebrate Communities in Wadeable Waters*. Kentucky Department for Environmental Protection, Division of Water, Frankfort, Kentucky. KDOW. 2008. *Methods for Assessing Biological Integrity of Surface Waters in Kentucky.* Kentucky Department of Environmental Protection, Division of Water, Frankfort, Kentucky.
Habitat Assessment	Provide a semi-quantified evaluation of the general habitat of the stream	Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish.* Second Edition. EPA 841-B-99-002. USEPA, Office of Water, Washington, D.C.
Hydrogeomorphic Sampling	Determine the degree to which streams are being effected by hydromodification including erosion rates, changes in the streambed, and position within the cycle of channel evolution in order to guide and prioritize remediation efforts	Bunte, Kristin; Abt, Steven R. 2001. *Sampling surface and subsurface particle-size distributions in wadable gravel-and cobble-bed streams for analyses in sediment transport, hydraulics, and streambed monitoring*. Gen. Tech. Rep. RMRS-GTR-74. Fort Collins,CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 428 p. Harrelson, C.C., C.L. Rawlins, and J.P. Potyondy. 1994. *Stream channel reference sites: An illustrated guide to field technique*. General Technical Report RM-245. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. 61p. Rosgen, D.L. 2008. *River Stability Field Guide*. Wildland Hydrology, Pagosa Springs, CO.
Grab sampling	Identify loading of water quality parameters to identify whether specific pollutants are causing impairments in the watershed	KDOW. Watershed Watch Water Chemistry Sampling Methods for Field Chemistry and Lab Analysis. http://www.lrww.org/training/chem-test.pdf KDOW. 2009a. *In-situ* Water Quality Measurements and Meter Calibration Standard Operating Procedure. Kentucky Department for Environmental Protection, Division of Water, Frankfort, Kentucky. DOWSOP03014 KDOW. 2010b. *Measuring Stream Discharge Standard Operating Procedure*. Kentucky Department for Environmental Protection, Division of Water, Frankfort, Kentucky. DOWSOP03019 KDOW. 2009b. *Sampling the Surface Water Quality in Lotic Systems*. Kentucky Department for Environmental Protection, Division of Water, Frankfort, Kentucky. DOWSOP03015 LaMotte. Dissolved Oxygen Water Quality Test Kit Instruction Manual. Code 7414 / 5860. LaMotte Company. Chestertown, Maryland. www.lamotte.com Price, David J. 2009. *Quality Assurance Plan (QAP) and Standard Operating Procedures (SOPs).* Lexington-Fayette Urban County Government Division of Water Quality Town Branch Laboratory. Kentucky Geological Survey Standard Operating Procedures. See Appendix B.

A7.2. Data Quality Indicators

When measurement performance or acceptance criteria can be stated in quantitative terms, they are called data quality indicators (DQI). The quality of field and analytical data is most often assessed in terms of the DQIs including precision, accuracy/bias, representativeness, comparability, completeness, and sensitivity.

A7.2.1. Precision

Precision is the measure of agreement among repeated measurements of the same property under identical or near identical conditions. It is usually calculated as a range, standard deviation, or relative percent difference (RPD). Relative percent difference will be the primary measure of precision for laboratory and field duplicates on this project, and is calculated as follows:

$$RPD(\%) = \frac{|S - D|}{\left(\frac{S + D}{2}\right)} \times 100$$

where:
S = first sample value (original result)
D = second sample value (duplicate result)

The project standards for precision on in-situ measurements and water quality grab samples are expressed in Table 5. The precision of in-situ measurements will be assessed by one duplicate measurement during each sampling event. The precision of the water quality grab samples will be measured by internal laboratory QC samples. In addition to internal laboratory QC, one field duplicate will be collected per sampling event. A field duplicate or field replicate sample is a sample taken from the same location as the 'regular' grab sample, at the same time. The sample is used to assess variability of environmental conditions at sampling sites.

For flow measurement, replicate measurements will be made to test the accuracy of the individual making the measurements. Replicate measurements are made by the same individual who made the original measurements and at the same cross section as the original, but with different horizontal locations (stations) across that cross-section. For example, if the original cross section had stations at even intervals (2, 4, 6, 8 etc.), the replicate measurement might have stations set at odd intervals (3, 5, 7, 9 etc.).

For benthic macroinvertebrate identification, ten percent of all sorting pans will be randomly checked by a second sorter to assure that samples have been picked thoroughly. Five percent of all identified samples will randomly be re-identified to insure QA/QC by a second taxonomist. Ninety percent or greater composition comparability (*e.g.,* abundance and richness) is the target success criteria. If there is less than 90 percent comparability between the taxonomists, then taxonomy must be reconciled by both taxonomists and a third taxonomist, if deemed necessary.

TABLE 6 – ACCEPTANCE CRITERIA FOR WATER CHEMISTRY AND *IN-SITU* MEASUREMENTS

Parameter	Units	Method	Accuracy (%R or ±)	Precision* (% RPD)	Sensitivity (Reporting Limit)
Dissolved Oxygen	mg/L	LaMotte	±1.5	20	0.5
Specific Conductance	µS/cm	Oakton	95-105	20	10
pH	SU	Watershed Watch	±0.5	20	NA
Water Temperature	˚C	Watershed Watch	±0.5	20	-5 to 45
Turbidity	Visual	Visual Observation	NA	NA	NA
Flow	cfs	DOWSOP03019	±0.05 ft/sec	N/A	0.01 ft/sec
Total Dissolved Solids	mg/L	EPA 160.1	95-105	20	10
Total Suspended Solids	mg/L	SM 2540 D	95-105	20	2
Total Alkalinity	mg/L $CaCO_3$	SM 2320 B	80-120	20	0
Hardness	mg/L $CaCO_3$	SM 2340 C	80-120	20	0
Escherichia coli	MPN/100mLs	SM 9221 E	N/A	20	1
Fecal Coliform	CFU/100mLs	SM 9221 F	N/A	20	1
Ortho-phosphorous as P	mg/L PO_4-P	EPA 365.1	80-120	20	0.05
Phosphorus, Total as P	mg/L PO_4-P	EPA 365.2	80-120	20	0.02
Ammonia as N	mg/L NH_3-N	EPA 350.1	80-120	20	0.05
Nitrate as N	mg/L NO_3-N	EPA 300.0	80-120	20	0.02
Nitrite as N	mg/L NO_2-N	SM 4500-NO_2 B	80-120	20	0.02
Total Kjeldahl Nitrogen as N	mg/L TKN-N	SM 4500-Norg C	80-120	20	0.5

** Indicates minimum laboratory precision for all parameters except in-situ measurements. For in-situ, this indicates field precision.*

For hydrogeomorphic sampling, the surveying precision of cross-sections and profiles shall be +/- 0.01 ft for vertical readings and +/- 0.1 ft for horizontal readings. The laser level precision shall be less than +/- 3.0 mm/30m. Precision for pebble count readings will be such that each data point measures within +/- 1 unit of the narrative particle description or +/- 0.5 phi units on the gravelometer.

A7.2.2. Accuracy

Accuracy is a measure of overall agreement between a measurement and a known value. Accuracy includes an evaluation of bias, which is a systematic or persistent distortion of a measurement process that causes errors in one direction. Accuracy is quantified by calculating the *percent recovery* (%R) of a known quantity of an analyte under a particular test method as follows:

$$\%R = \frac{V_m}{V_t} \times 100$$

where:
V_m = measured value (determined by analysis)
V_t = true value (as calculated or certified by a manufacturer)

No water quality field samples will be collected in order to evaluate accuracy. However, internal laboratory QC samples will be analyzed to determine if the project standards, listed in Table 4, are met. For benthic macroinvertebrate samples vouchers are collected

to ensure accuracy. Field photographs are used to document accuracy for habitat assessment.

For the conductivity survey, the GPS units used to document locations shall be accurate to at least 20 feet, and the latitude and longitude shall be recorded in decimal degrees to six decimal places in datum NAD83.

A7.2.3. Representativeness

Representativeness is the degree to which data accurately and precisely represent an environmental condition. Representativeness is largely a product of proper selection of sampling sites within the watershed and proper execution of the methodology. For instance, per the sampling method, grab samples are to be collected from the thalweg and not from the bank in order to ensure representativeness. Representativeness is also ensured by collection under the specified sampling conditions. Representative dry weather sampling conditions are defined by an antecedent period of 5 days during which there is no rainfall. Representative wet weather sampling conditions are defined by a period of at least 72 hours of dry weather preceding a rain event with a total accumulation of at least 0.1 inch. Sampling during 'wet-weather' will occur on the hydrographic rise. Representative conditions for macroinvertebrate sampling are established by the respective index periods for sampling. In addition, macroinvertebrate samples will not be collected during periods of excessively high or low flows or within two weeks of a known scouring flow event.

Other anomalous conditions or unusual land uses at the time of sampling will be recorded in the field notebook.

A7.2.4. Completeness

Completeness is a measure of the amount of valid data needed to be obtained from a measurement system. The completeness goals for each of the sampling types are summarized in Table 7. Dry weather is expected to produce no flow conditions at several sites during the sampling period.

TABLE 7 – COMPLETENESS GOALS OF SAMPLING ACTIVITIES

Sampling Activity	Completeness Goal
Karst Hydrograph Characterization	At each site, a dry-weather event and measurements from the first-flush to past the peak flow during a wet-weather event
Conductivity Survey	Minimum of 500 sites with at least one in each of the 24 stream segments
Macroinvertebrate Collection	All six sites
Habitat Assessment	Minimum of one site from each of the 24 stream segments
Hydrogeomorphic Sampling	All nine sites – two surveys
Grab sampling	Five samples in 30 days for *E.coli.* At least two 'dry-weather' and two 'wet-weather' events at all sites; no flow conditions are expected during dry ant hot weather conditions at several headwater sites

A7.2.5. Comparability

Comparability is a term that expresses the measure of confidence that one data set can be compared to another and can be combined for the decision to be made. Comparability may be assessed by comparing sampling methodology, analytical methodology, and units of reported data. The standards of quality established in this QAPP are consistent with the previously collected data in the Wolf Run watershed collected under separate QAPPs. All data to be utilized in the generation of the watershed based plan source and loading determinations will have been generated under an approved QAPP.

A7.2.6. Sensitivity

Sensitivity is the capability of a method to discriminate between measurement responses representing different levels of the variable of interest. Sensitivity is particularly important for ensuring that the measurement levels are sufficient to detect whether particular pollutants are present at levels that may cause impairment to the designated use. For grab sampling and *in-situ* measurements, the sensitivity levels necessary for this program are specified in Table 6. For macroinvertebrate sampling, all organisms are to be identified to the lowest possible taxonomic level possible in order to properly calculate the associated metrics.

A8. Training Requirements

All volunteers involved in the sampling for this project shall have successfully completed the training workshops lead by a trainer registered with the KDOW Watershed Watch Volunteer Database as administered by the Watershed Management Branch. The training will consist of four sessions that will cover habitat assessments, grab sample collection, *in-situ* measurements, and the requirements of this QAPP. Powerpoint presentations to be used in this training are available at http://www.lrww.org/training/. The training will be organized and conducted by the FOWR and their Science Advisors. The FOWR Sampling Coordinator will maintain documentation of the volunteer sampler training through the Participant Agreement Form (Appendix A).

A9. Documentation and Records

In order to provide quality data that meets the project objectives, traceability and maintenance of documentation and records is essential. All records relating to the collection, analysis, or reporting of data associated with the project shall be made available upon request by KDOW or LFUCG. A summary of such documentation is included below.

A9.1. Field Documentation and Records

Field records will include all data recorded in the field including completed field datasheets, field logbooks, monitoring records, and chain of custody sheets. All data will be recorded using black or blue indelible ink, and it is recommended that waterproof paper be used where feasible. Mistakes on field data sheets will be crossed out with one line (so the information is still discernible), with the initials and date of the person making the correction. The correct information should then be recorded legibly on another line, or above or below the original info. If a separate sheet is necessary for new

information, the original sheet should be attached to the new sheet, and initialed and dated.

The following field documents shall be used in this project:

- Site Characterization Form
- High Gradient Stream Data Sheet for Habitat Assessment
- Conductivity Survey Field Data Sheet
- Pebble Count Field Data Sheet
- Water Quality Chain-of-Custody
- Macroinvertebrate Chain-of-Custody
- Sample Labels
- Field "Rite in the Rain" Notebook
- Field "Reference Reach" Notebooks
- Field Meter Calibration Logs

Copies of these documents are found in Appendix A, where applicable.

All raw data collected in the field will ultimately be submitted in the chemical or biological data package. However, all field notes, including the location and frequency of QC sampling, *in situ* measurements, and calibration and maintenance logbooks will be retained for the duration of the grant period.

Where possible, all field *in situ* measurements will be recorded on the datasheet or chain-of-custody. However, if necessary, results or notes may be maintained in a field notebook. Equipment calibration and maintenance logs will be documented and recorded per procedure specifications.

A9.2. Laboratory Documentation and Records

Chemical laboratories are required to maintain a current Quality Assurance Manual documenting all aspects of their quality system including control of Standard Operating Procedures (SOPs) and datasheets. Documents issued as part of the quality system will be reviewed and approved by authorized personnel. A master list identifying the current revision and distribution of documents in the quality system will be used to ensure that invalid and obsolete documents are not used. Quality system documents will be uniquely identified by the date of the last revision, issuing authority, and the total number of pages or a mark indicating the end of the document. The *Lexington-Fayette Urban County Government Division of Water Quality Town Branch Laboratory Quality Assurance Plan (QAP) and Standard Operating Procedures (SOPs)* (Price 2009) and the Kentucky Geological Survey's *Standard Operating Procedures* located in Appendix B will control the chemical laboratory document control.

Laboratories will be required to document the analysis of all quality controls associated with the analysis of the collected samples such that the entire data package, along with a narrative description of the results and a list of all data qualifiers, may be provided to the

KDOW and LFUCG upon request. Thus, the laboratories will retain all data associated with the sample analysis for the duration of the grant period.

Third Rock's macroinvertebrate identification laboratory will follow laboratory protocols for benthic macroinvertebrate sample processing, identification and data reporting per KDOW (2009b, 2008) with the following exceptions:

- All samples will be logged into Third Rock's Macroinvertebrate Laboratory Information Management System (MacLIMS) upon receipt.
- Sample identification date will be maintained in MacLIMS.
- Taxonomic QA/QC dates (if applicable) will be noted on individual QA/QC forms and maintained electronically in the Project File.
- Initials of the applicable party completing each task associated with sorting, identification, or quality control will be noted electronically in MacLIMS or on associated QA/QC forms.
- QA checks will be documented on applicable forms and maintained in associated project files. These forms include the Macroinvertebrate Sample Sorting Efficiency Form, Macroinvertebrate Sample Taxonomy Precision Form, and Macroinvertebrate Sample Taxonomic and Enumeration Efficiency Form (Appendix A).

Completed chain(s)-of-custody and sample labels will also be controlled throughout the analysis process, and completed chain(s)-of-custody will be submitted in the data package. The laboratory will retain all data associated with the sample analysis for the duration of the grant period.

Expected turn-around time for the laboratory analysis is 30 days. Chemical laboratory reports with data quality review by the Project QA Manager will be submitted to the project team within 60 days of sample delivery to the laboratory. The chemical laboratory data package will include the laboratory results, completed chain(s)-of-custody, lists of qualifiers associated with the data, and a report of the quality control results.

The macroinvertebrate report data package will include a list of the identified species, metric calculations, habitat assessment scores, photographs, completed chain(s)-of-custody, and a data analysis report. This report will be submitted to KDOW and LFUCG prior to December 31, 2011.

A9.3. Quality Documentation and Final Reports

The most recent version of this QAPP will be distributed to all parties listed on the distribution list after the QAPP has been reviewed and approved. The QA Manager is responsible for ensuring that all applicable parties perform documented reviews of the QAPP. If, because of deviations in the QAPP, revisions are required, the QA manager shall ensure that all parties review the revised version. The current revision and the date of the revision shall be documented in the upper right corner of the QAPP pages. The QAPP shall be redistributed after all parties have reviewed the document.

As a result of this project, multiple final reports will be used to document the findings of the monitoring performed under this QAPP. Monitoring reports will be submitted to the KDOW and LFUCG in hardcopy upon request. Electronic data will be presented in Adobe Acrobat, Microsoft Word, and/or Microsoft Excel depending on the data type. These final reports are as follows:

- Karst Hydrograph Characterization in the Wolf Run Watershed
- Assessment of Habitat and Macroinvertebrates in the Wolf Run Watershed
- Conductivity Survey of the Wolf Run Watershed
- Hydrogeomorphic Assessment of the Wolf Run Watershed
- Wolf Run Watershed Monitoring Report
- Wolf Run Watershed Based Plan
- Final Project Report

The Wolf Run Watershed Monitoring Report will include an evaluation of the quality assurance and will compare the data produced under the water quality monitoring to the data quality indicators listed herein. The Wolf Run Watershed Based Plan will be developed in accordance with the KDOW's *Watershed Planning Guidebook for Kentucky Communities* (2010) and meeting the USEPA's nine key elements for watershed based plan. The Final Project Report will meet the requirements of the KDOW *Project Final Report Guidelines for Clean Water Act §319(h)-Funded Projects* (2004).

In addition to these reports, quarterly Section 319(h) Nonpoint Source Project Progress Report will be submitted to the KDOW to document the progress on the project milestones.

SECTION B. - DATA GENERATION AND ACQUISITION

B1. Sampling Process Design

As previously mentioned, this QAPP addresses six different monitoring types. A summary of the sampling locations and rationale behind the monitoring types is provided below.

B1.1. Sampling Site and Reach Locations

Exhibits 1 and 2 indicate the locations of the permanent sampling sites on aerial and geologic quadrangle mapping. Table 8 describes the location of each sampling site and whether monitoring for the karst hydrograph characterization, macroinvertebrate collections, hydrogeomorphic sampling, or water quality monitoring will be performed at these sites.

TABLE 8 – SAMPLING LOCATIONS

Site Name	Stream	Location	Directions	Latitude	Longitude	Monitoring Type			
						Karst	Macro	Geomorph	WQ
W1	Wolf Run	Old Frankfort Pike	Downstream of Old Frankfort Pike northbound prior to roundabout at Alexandria Dr.	38.067303	-84.554182	X	MS4	X	X
W2	McConnell Branch	Prestons Cave	Park to the right of the Assembly of God Church on Dunkirk Drive. Access site through grass lot next to the church to spring on the left.	38.057333	-84.542169	X	X	X	X
W3	Wolf Run	Valley Park	About 50 feet downstream of Cambridge Drive Bridge. Park in Cambridge Park Appartments parking lot.	38.053742	-84.550782				X
W4	Vaughn's Branch	Valley Park	About 30 feet upstream of confluence with Wolf Run. Sample upstream of the sanitary sewer manhole cover. Park in Cambridge Park Appartments parking lot.	38.054904	-84.549624	X	X	X	X
W5	Cardinal Run	Devonport Dr	Upstream of Devonport Drive, west of Alexandria Dr. Park in Pleasant Cove Apartments lot to left of bridge, take sample at rock upstream of bridge.	38.048594	-84.553867				X
W5A	Cardinal Run	Parkers Mill Rd	Park on Cross Keys Road, just off of Parkers Mill Road. Sample reach as diverges from curbside.	38.043212	-84.557131		X	X	
W6	Wolf Run	Wolf Run Park	Take Roanoke Dr off of Alexandria Dr. past 7 Pines Dr. Park uphill of cement slab in greenway, sample at path crossing creek.	38.045274	-84.550661	X	X	X	X
W7	Vaughn's Branch	Pine Meadow Park	Turn right onto Tazwell Drive off Mason Headly Road. Travel to the end of the road. Walk below the playground to sample.	38.044927	-84.536148			X	X
W8	Vaughn's Branch	Picadome Golf Course	Park at lot to right of Parkway Drive in front of Picadome Golf Course. Site is north of the clubhouse, midway between the bridges between holes 4 and 5	38.037453	-84.525057			X	X
W9	Wolf Run	Faircrest Drive	At terminus of Faircrest Dr south of Lynn Dr. Sample upstream of confluence with Springs Branch.	38.029954	-84.537091	X		X	X
W10	Springs Branch	Faircrest Drive	From W9, cross Wolf Run at foot bridge and sample upstream on Spring Branch.	38.029855	-84.537196				X
W11	Big Elm Tributary	Harrodsburg Road	Park at end of parking lot behind the Harrodsburg Rd Fire Station. Access just north of power transformer at edge of adjacent parking lot.	38.031245	-84.526027	X	X	X	X
W11A	Big Elm Tributary	Picadome Golf Course	Park at lot behind the Picadome Golf Course clubhouse. Measure discharge at confluence of surface runoff with Vaughn's Branch if flow is present.	38.037494	-84.527095	O			
W12	Wolf Run	Lafayette Parkway	Sample about 50 feet below bridge at Rosemont Garden	38.022932	-84.528581		X		X

NOTE: "X" indicates permanent sampling location. "O" indicates measurements will occur if flow is present. "MS4" indicates that sampling is scheduled but under the MS4 permit and not this project.

For the conductivity survey and watershed habitat assessments, the watershed has been divided into twenty-four stream segments as shown in Exhibit 3 and summarized in Table 9.

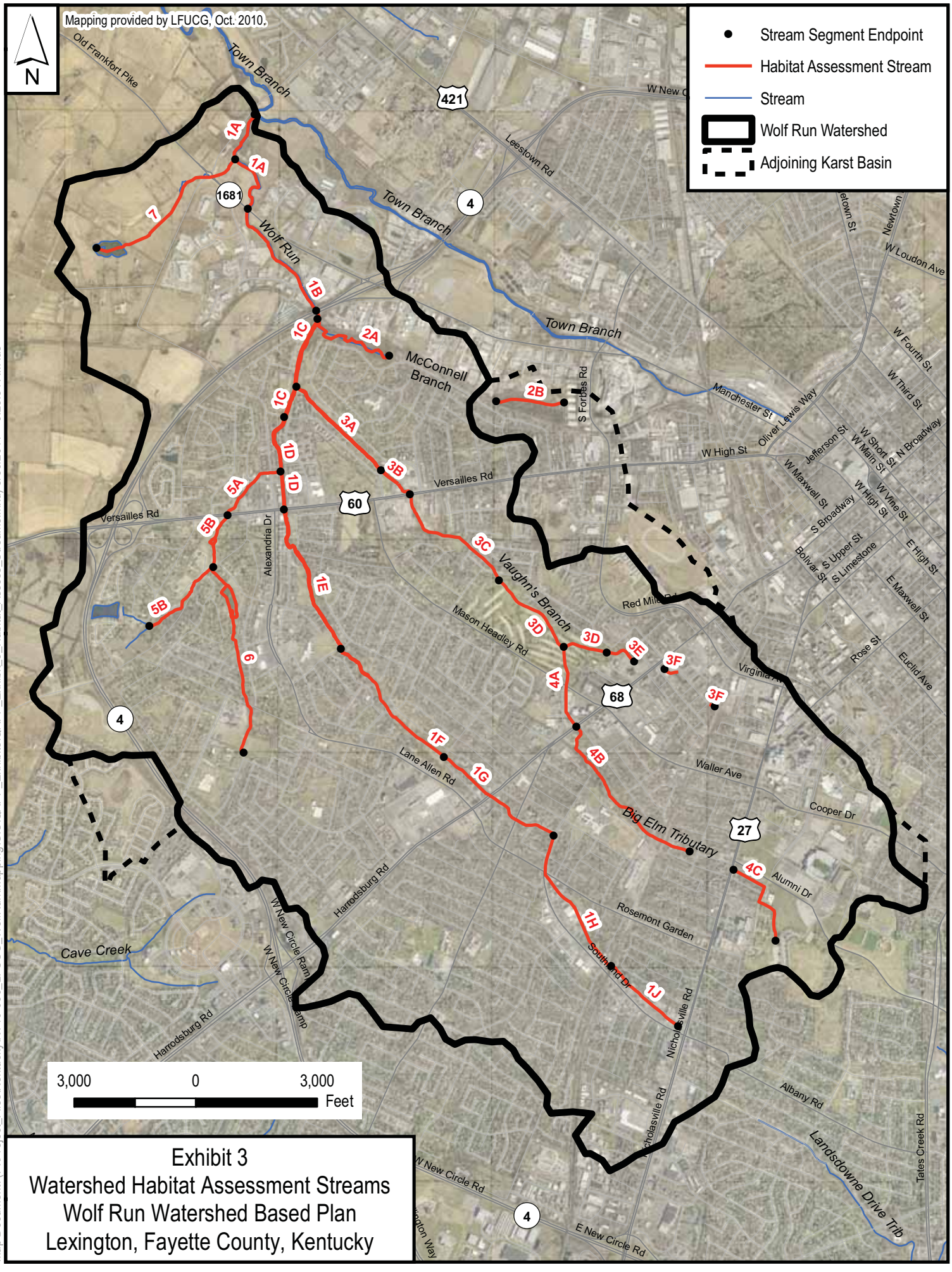
TABLE 9 – HABITAT ASSESSMENT STREAM SEGMENTS

Segment ID	Stream	Stream Segment
1A	Wolf Run	Mouth to Old Frankfort Pike
1B	Wolf Run	Old Frankfort Pike to New Circle Rd
1C	Wolf Run	New Circle Rd. to Cambridge Dr
1D	Wolf Run	Cambridge Dr to Versailles Rd
1E	Wolf Run	Versailles Rd to Appomattox Rd
1F	Wolf Run	Appomattox Rd to Faircrest Dr
1G	Wolf Run	Faircrest Dr to Lafayette Pkwy
1H	Wolf Run	Lafayette Pkwy to railroad tracks north of Southland Dr
1J	Wolf Run	Railroad tracks to Nicholasville Rd
2A	McConnell Branch	Wolf Run to Preston's Cave Spring
2B	McConnell Branch	McConnell Springs Branch through Stormwater Structure
3A	Vaughn's Branch	Wolf Run to Oxford Circle
3B	Vaughn's Branch	Oxford Circle to Versailles Rd
3C	Vaughn's Branch	Versailles Rd to Summerville Rd/Golf Course Fence
3D	Vaughn's Branch	Picadome Golf Course
3E	Vaughn's Branch	Picadome Golf Course Fence to Gibson Ave Culvert
3F	Vaughn's Branch	Simpson Ave to railroad tracks
4A	Big Elm Tributary	Sinkhole on Picadome Golf Course to Harrodsburg Rd
4B	Big Elm Tributary	Harrodsburg Rd to railroad tracks via Bob-O-Link Dr
4C	Big Elm Tributary	Nicholasville Rd to behind Central Baptist Hospital
5A	Cardinal Run	Wolf Run to Versailles Rd
5B	Cardinal Run	Versailles Rd to End of Chinquapin Ln
6	Gardenside Tributary	Upstream of Parkers Mill Rd
7	Unnamed Tributary	Wolf Run under Alexandria Dr and Old Frankfort Pike to pond

B1.2. Sampling Design Rationale

B1.2.1. Karst Hydrograph Characterization

The Wolf Run watershed has significant karst development, which must be considered during loading calculations because it can influence the decision making process during development of the action plan. In particular, local karst deviation from surface watershed boundaries adds drainage area to Wolf Run. Based on dye traces, a substantial fraction of both the Vaughn's Branch and main stem of Wolf Run sub-watersheds are captured by the Prestons (McConnell) Spring Basin (Recker and Meiman, 1990 and Spangler, 1992). During base flow and drier conditions most of the surface water in the karst-influenced fractions of these sub-watersheds is directed to Prestons Spring. During high flow conditions the surface component of the discharge becomes greater as the karst system conduit limits are approached. To determine the influence of the karst system, storm event and base flow gaging of key locations will be conducted to determine the discharge and the nature of the hydrograph.


Mapping provided by LFUCG, Oct. 2010.
N
Stream Segment Endpoint
Habitat Assessment Stream
Stream
Wolf Run Watershed
Adjoining Karst Basin
Town Branch
Wolf Run
McConnell Branch
Vaughn's Branch
Big Elm Tributary
Cave Creek
Mason Headley Rd
Lane Allen Rd
Versailles Rd
Harrodsburg Rd
Leestown Rd
Old Frankfort Pike
Alexandria Dr
Rosemont Garden
Nicholasville Rd
Scholasville Rd
Albany Rd
Landsdowne Drive Trib
Tates Creek Rd
Waller Ave
Cooper Dr
Alumni Dr
Southland Dr
Red Mile Rd
W High St
W Main St
W Short St
W Vine St
E High St
E Maxwell St
W Maxwell St
W Loudon Ave
W Fourth St
W Third St
Euclid Ave
Rose St
Manchester St
Oliver Lewis Way
Jefferson St
S Broadway
N Broadway
Bolivar St
S Upper St
S Limestone
S Forbes Rd
Newtown
421
1681
4
60
68
27
1A
1B
1C
1D
1E
1F
1G
1H
1J
2A
2B
3A
3B
3C
3D
3E
3F
4A
4B
4C
5A
5B
6
7
3,000
0
3,000
Feet
Exhibit 3
Watershed Habitat Assessment Streams
Wolf Run Watershed Based Plan
Lexington, Fayette County, Kentucky
Map Document: (P:\Project_Files\Kentucky\KY10-030_LFUCG_WolfRunMapping\GIS\QAPP_Exhibits\QAPP_Exhibit_3_Habitat_Assess_Streams.mxd) 3/30/2011 -- 9:29:34 AM las

The six gaging locations in the watershed allow for the evaluation of the discharge at the mouth of the watershed (W1), Prestons Spring (W2), Vaughn's Branch tributary (W4), Wolf Run upstream of Cardinal Run (W6), Wolf Run at the edge of the karst basin (W9), and Big Elm Tributary which flows into the sinkhole at Picadome (W11). In the event that the capacity of the sinkhole downstream of W11 is exceeded and surface water flows from Big Elm Tributary into Vaughn's Branch, this surface flow will be measured at W11A in order to measure the input into the karst system at this site.

B1.2.2. Conductivity Survey

Specific conductance was recently listed as a cause of impairment in the Wolf Run watershed. Although specific conductance or conductivity has been analyzed during several studies and the FOWR conducted a broad study of conductivity levels in the watershed, a subsequent study under more normal flow conditions will aid in identifying inputs and problem areas. Conductivity is a measure of water's ability to conduct an electric current, and it indicates the concentration of dissolved ions in the water. Rapid changes in the conductivity of a waterbody can indicate groundwater input, catchment geology, or pollution.

B1.2.3. Benthic Macroinvertebrate Collection

Benthic macroinvertebrate sampling integrates months or even years of water quality impacts as well as the cumulative effects of multiple stressors and pollutants instead of particular individual stressors. The KDOW uses biological indicators to determine the use attainability of a water of the Commonwealth as it relates to KDOW's narrative water quality standards. Biological assessment will provide a more accurate evaluation of water quality health in the watershed. The six macroinvertebrate sites are located on the tributaries of Wolf Run and in its headwaters to evaluate the macroinvertebrate communities in the headwaters for comparison to the data collected over multiple years near the mouth of the watershed.

B1.2.4. Watershed Habitat Assessments

Habitat assessments will be used to supplement biological and physicochemical data when determining the overall health of the stream reach and stream-use designation. Additionally, habitat assessments will serve as a baseline to document physical changes that occur over time and to identify potential areas for BMP implementation. The watershed was divided into 24 stream segments in order to ensure that assessments are collected from all representative reaches.

B1.2.5. Hydrogeomorphic Assessment

The process of development within the Wolf Run watershed has affected the stream by altering watershed hydrology and sediment-transport patterns. The large amount of impervious surface has greatly reduced the capacity of the watershed to capture and filter rainfall. Higher runoff rates mean that runoff reaches the stream channels more quickly (flashier flows) and peak discharge rates are higher compared to an undeveloped watershed for the same size rainfall event. These effects are known as hydromodification. Hydromodification can also be direct modification of a stream (for purposes of flood control, navigation, sediment control, infrastructure protection, etc.), such as

channelization, armoring, and removal of riparian vegetation. Channel erosion and bank failure is often caused or exacerbated by hydromodification activities.

Nine hydrogeomorphic monitoring sites have been preliminarily identified throughout the watershed where quantitative data will be collected to measure channel change in representative reaches. It is expected that the nine hydrogeomorphic monitoring sites represent stream reaches that are susceptible to the effects of hydromodification, are in need of management to stop further degradation, and would be good locations to implement remediation. Assessment will include a series of spatially integrated, high-resolution cross-section and longitudinal profile surveys and streambed substrate evaluation to determine the extent of the effects of hydromodification in the Wolf Run watershed. Effects of hydromodification that may be revealed by the assessment include degree of bed and bank erosion, sedimentation, and habitat loss. The relative potential for improvement will also be qualitatively assessed based on the lack of obvious physical constraints in a reach, position in the landscape, or position in the watershed.

B1.2.6. Water Quality Monitoring

The objective of the water quality monitoring is to provide sufficient temporal and geographic data to evaluate the sources and loadings of water quality pollutants. The sampling period of ten months was selected in order to evaluate at least one sample from all seasons. The twelve sampling stations were selected in order to evaluate the relative contributions of the stream reaches throughout the watershed.

Flow will be measured on the receiving streams because it is a component of the formula for calculating loading of pollutants in the watershed. Dissolved oxygen, temperature, and pH will be assessed as basic measurements for describing the health of the stream and evaluating applicable water quality standards. *E. coli* and fecal coliform will be used to assess health risk due to waterborne pathogens. Specific conductance and total dissolved solids will be used to assess dissolved ions levels present in the watershed. Turbidity and TSS will be recorded to assess the suspended solid levels for impacts to stream biota due to increased turbidity, siltation, and other effects. Ortho-phosphorus, total phosphorus, nitrate, nitrite, and TKN will be assessed to identify imbalances which may cause eutrophication and impacts to stream biota. Ammonia will be assessed to evaluate levels for toxicity to plants, animals, and humans. Alkalinity and hardness will be assessed to measure the buffering capacity of the water against rapid pH changes.

Because *E. coli* levels will be evaluated against the geomean criteria, it is necessary to collect five samples in 30 days for this project. For other parameters, at least two 'dry-weather' and two 'wet-weather' events will be sampled at all sites in order to adequately characterize the loadings geographically. It is expected that no flow conditions will be observed in the watershed during the sampling period.

B2. Sampling Methods

B2.1. Sampling Equipment

Equipment to be utilized in sampling is listed in Table 3, Sampling Equipment Summary. Samples are to be collected and preserved according to the specifications in Table 10.

TABLE 10 – SAMPLE PRESERVATION AND HOLD TIME

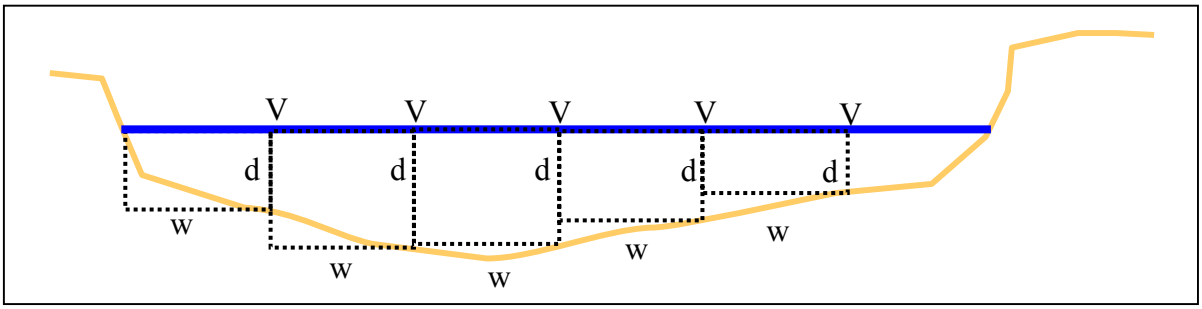
Parameter	Analysis Method	Maximum Holding Time	Sample Container	Sample Preservation	Deliver To
E. coli	SM 9221 E	6 hrs	Sterilized Plastic, 4 oz	Cool <6°C, $Na_2S_2O_3$ (No Cl_2)	LFUCG Town Branch Laboratory
Fecal Coliform	SM 9221 F	6 hrs	Sterilized Plastic, 4 oz		
Ammonia	EPA 350.1	28 days	Plastic, 8 oz	Cool <6°C, $Na_2S_2O_3$ (No Cl_2), H_2SO_4 to pH <2	
Alkalinity	SM 2320 B	14 days	Plastic, 32 oz	Ice to <6°C	
TSS, TDS, Total Hardness, Nitrite	SM 2540 D, EPA 160.1, SM 2340 C, SM 4500-NO_2 B	7 days			
Ortho-phosphorus	EPA 365.1	28 days	Plastic, 8 oz	Field Filter, Cool <6°C, H_2SO_4 to pH <2,	Kentucky Geological Survey Laboratory
Total phosphorus, TKN	EPA 365.3, SM 4500-Norg C	28 days	Plastic, 32 oz	Cool <6°C, H_2SO_4 to pH <2	
Nitrate	EPA 300.0	7 days	Plastic, 8 oz	Ice to <6°C	
Macroinvertebrate samples	KDOW 2009c	None	1 Liter wide mouth plastic	95% ethanol	Third Rock Macroinvertebrate Laboratory

B2.2. Sampling Methods

B2.2.1. Karst Hydrograph Characterization

Because the karst drainage collected by the Prestons Spring Basin emerges as a spring and surface water, only surface water discharge methods will be used to characterize the hydrograph of the karst system. Surface water discharge (Q) will be calculated using two variables, flow area (A) and water velocity (V), according to the equation: $Q = AV$. However, because the velocity is variable across a stream cross-section, the flow area and velocity must be measured in intervals across the stream and summed as shown in Figure 2. The flow area of each interval is the product of the width (w) and depth (d) for that interval. The velocity will be measured for each of these areas.

FIGURE 2 – MEASUREMENT OF DISCHARGE THROUGH SUB-SECTIONAL MEASUREMENTS



Note: Stream cross-section showing intervals where water depth and velocity are measured. Flow will be calculated for each "box" (flow area for each box is d * w) and summed to obtain the flow for the entire stream.

Flow measurements will be conducted according to the KDOW's *Measuring Stream Discharge Standard Operating Procedure* (KDOW 2010b), as described below. Under this procedure, a tape measure of at least 100 feet is stretched across the stream so that it is taut and perpendicular to the stream flow lines. The tape measure is located directly above the cross-section to be measured and must not touch the water surface.

Identify the starting edge as either left edge of water (LEW) or right edge of water (REW) when facing downstream. Determine the approximate width of the stream with active stream flow, being sure not to include slack water areas. Hence, the edge of slack water areas will be considered the edge of the stream.

Discharge measurements are taken at several verticals, defined as a point along the cross-section where water velocity is measured at a defined depth (or depths). Twelve to twenty verticals will be targeted for streams <20 feet wide, whereas twenty to thirty verticals will be targeted when stream width is >20 feet. To calculate the approximate spacing of verticals, divide the stream width by the number of desired verticals. Importantly, the stream discharge computed using the average velocity in one vertical shall not exceed 10% of the total stream discharge. Therefore, it may be necessary to space verticals more closely together in areas that are deeper or that have a greater velocity than the majority of the stream. Conversely, the spacing of verticals may be farther apart in areas that are shallower or have lower velocity compared to the majority of the stream. Uniform spacing across the tape measure will only be used if the stream is of relative uniform depth and velocity regimes.

Although vertical spacing can vary, verticals will never be spaced less than 0.2 feet apart. As a result of this minimum spacing, small streams with a flowing width of less than 2.2 feet will have less than 12 verticals and can have as few as one vertical during very low stream flow.

A standard top-setting wading rod will be used to measure water depth and confirm the proper location of the flow meter sensor within the water column. The wading rod will be adjusted to the appropriate depth, which is marked in 0.1-foot increments along the rod. It

is appropriate to further estimate depth to the 0.05-foot increment level, despite the wading rod not being marked to this level. When water depth is ≤ 2.5 feet, velocity is measured at 0.6 of the depth below the water surface at each vertical. A standard top-setting wading rod will automatically adjust the probe to this height. When water depth is ≥ 2.5 feet, discharge is measured at 0.2 and 0.8 of the total depth below the water's surface at each vertical. To set the rod at the 0.2-depth, position the setting rod at half the water depth. To set the rod at the 0.8-depth, position the setting rod at twice the water depth. An average of these two readings will be used as the average velocity for the vertical.

The wading rod will be held perpendicular to the water surface, and the instrument will be parallel to the stream flow. The individual making the measurements will stand at least 1.5 feet away from the wading rod and 3 inches downstream of the tagline in a way that alters the stream flow as little as possible. Rocks, logs, or other obstructions will not be moved during the measurement process as this may cause the stream flow to change in an area of the stream where velocity has already been measured. Once the process of measuring velocity has begun, the stream will not be altered.

Record the location of the starting edge on the field data sheet (LEW or REW). If the starting edge has a water depth, record this. No velocity measurements will be made at the starting or ending edges. Facing upstream, place the wading rod behind the tape measure at each vertical and record the location and stream depth. Velocity readings will be averaged over a time period of 25s – 45s, depending on in-stream conditions. If the water depth is ≥ 2.5 feet at a station, indicate the depth (0.2 or 0.8) associated with each of the two velocity measurements. Record the ending edge (LEW or REW) as well as the depth and velocity, if these exist.

If the stream cannot be safely waded or if a flow meter is not accessible, floats can be used to estimate stream velocity needed for stream discharge computation. All measurements using this procedure should be flagged as estimated on field data sheets and on final data reports. The following steps are used in these conditions:

1. Find a long, relatively straight section of stream that allows a travel time of 20 seconds. A shorter time can be used if these conditions cannot be met.
2. Select two cross sections along the reach; one at the top and one at the bottom.
3. Measure the width of the stream at the cross sections and in a few areas between the cross sections to obtain an average width. If the stream is not wadeable, estimate the width. Record the width on the field data sheet.
4. Estimate how far an object will float in 20 seconds and stretch a tagline along the stream bank to account for that distance. A distance of 30-50 feet is ideal. A shorter run length may be used if these conditions cannot be met.
5. Based on the width, divide the stream into 2-3 longitudinal profiles. Measure or estimate the depths at these profiles. On the field data sheet record the nearest bank (REW/LEW) as 0. Record the farthest bank as the total width of the stream.

6. Have one person stand at the starting point on the tagline and a second person stand at the point designated as the end of the run. The person at the end of the run should use a stopwatch that can measure to tenths of a second.
7. The person at the starting point will throw a floating object (large stick, orange, hedge apple, etc.) just upstream of the top cross section within the first longitudinal profile area. When the object crosses the upstream cross section, the person will say "start" and the person at the end of the run will start the timer.
8. When the object crosses the downstream cross section, the person with the timer will stop the timer.
9. Record the distance the object traveled and the number of seconds, to the tenths of a second, the object took to travel that distance.
10. Repeat Steps 6-9 for the remaining profiles.

Velocity and flow area measurements must manually be recorded in a bound field book, or on other appropriate field data sheets, using indelible, waterproof ink and waterproof paper. Discharge values are to be calculated in the office according to the equations specified in KDOW 2010b.

In addition to the discharge measurements collected, temporary water level gages (pressure transducers with data loggers) will be installed at each of the six gaging stations. These gages will be installed inside PVC pipe attached to a firmly anchored stake or permanent instream structure with an elbow facing downstream to eliminate bias from velocity-based pressures. If water levels become elevated enough to make streams unwadeable and flow measurements unfeasible, the water level recorded by the loggers may be useful in estimating stream discharge. The water level recorded by the loggers could be used along with stream cross-section measurements and an estimation of surface velocity (*i.e.* 'float' method) to estimate discharge during any un-wadeable parts of the wet event.

The discharge data collected in this study will be used to improve modeling of the watershed discharge based on the USGS gage and land use to incorporate the redirection of the flow through the karst system. The results will be summarized in the "Karst Hydrograph Characterization in the Wolf Run Watershed" final report due by May 31, 2012.

B2.2.2. Conductivity Survey

Trained volunteer samplers under the direction of the FOWR Sampling Coordinator will conduct the conductivity survey. The Sampling Coordinator will schedule the survey such that flow conditions at the USGS gage at the mouth of Wolf Run are between 0.5 and 5 cfs. In order to limit the temporal variations in the conductivity levels, efforts will be made to conduct the entire survey within a one-week period unless interrupted by precipitation. In the event of a precipitation event, the Sampling Coordinator will reschedule the remaining sampling a minimum of 72 hours after the precipitation has ceased.

Each volunteer will be equipped with a GPS unit, digital camera, conductivity meter, thermometer, and Conductivity Survey Field Datasheets (Appendix A). Volunteers will each be assigned stream segments as shown in Table 9 and Exhibit 3. Before and after monitoring activities, the volunteer shall calibrate the conductivity meters using a standard of known value. The standard value, initial calibration reading (prior to monitoring) and final calibration reading (after monitoring is completed) shall be recorded on the datasheets along with the meter identification number (serial number). Calibration and measurements with the conductivity meter shall be performed according to the specifications in the instruction manual (see Appendix B).

Volunteers will begin the survey in the upstream portion of these reaches and work downstream recording the time of measurement, latitude and longitude (NAD83 decimal degrees), conductivity, temperature, and additional observations (including anomalous conditions), if applicable, at each site. Photographs will be taken to document unusual conditions with the photograph numbers indicated on the datasheet. Datasheets will be used to document only one stream reach and monitoring day. If multiple datasheets are necessary to cover a given stream segment, the order of these datasheets must be indicated in the "Segment ID" by a dash and number value. For instance, if two datasheets were used on Wolf Run segment 1A, these would be labeled 1A-1 and 1A-2 in the "Segment ID" on the datasheet.

Datasheets and photos will be submitted to the FOWR Sampling Coordinator to be compiled into a Microsoft Excel database. The FOWR Sampling Coordinator will submit the electronic database, scanned copies of the field datasheets, and an electronic photo library to Third Rock by November 30, 2011. Third Rock will use the data to produce GIS images of the watershed, indicating hotspots. A final report "Conductivity Survey of the Wolf Run Watershed" discussing the methods, results, and conclusions based on the monitoring will be completed by December 31, 2011.

B2.2.3. Benthic Macroinvertebrate Collection

Sampling for benthic macroinvertebrates will be conducted according to the KDOW's *Methods for Sampling Benthic Macroinvertebrate Communities in Wadeable Waters* (KDOW 2009c). All sites are headwater sites except W1, which is sampled for macroinvertebrates under the MS4 permit and not under this project.

A collection event consists of a composited semi-quantitative sample and a composited multi-habitat sample. Semi-quantitative samples will be collected from a known area in order to indicate the macroinvertebrate community in the most productive habitat in the stream niche (*i.e.,* riffle). Multi-habitat samples are intended to identify other taxa present in the stream that may not be collected in the semi-quantitative sampling. These two sample types must be kept separate for effective diagnosis of impairment. A summary of the collection techniques used for wadeable and headwater streams is shown in Table 11 and further described in the following sections.

It is important to keep in-stream habitat intended for benthic macroinvertebrate sampling intact and undisturbed until the single and multi-habitat samples have been collected.

Therefore, field personnel must avoid walking through areas designated for collection of benthic macroinvertebrates until sampling has been completed. Failure to use caution could result in sample degradation.

After collections are completed, large sticks and leaves will be washed in the field, inspected for organisms and discarded. Rocks will be elutriated and hand washed into a bucket and 600µm sieve. This process will be repeated until a manageable amount of debris and organisms (relative to size of sample container) can be preserved for laboratory sorting. Samples may be partially field picked using a white pan and fine-tipped forceps. The sample container will be preserved with 95% ethanol. While at the sampling location, all macroinvertebrate samples will receive a label. The label may be placed in the sample jar (labels placed in the jar will be written in No. 2 pencil on waterproof paper) and written directly on some portion of the jar. The label will include the site number, if known, stream name, location, county, date sampled and the collector's initials.

TABLE 11 – SUMMARY OF SAMPLING METHODS FOR MACROINVERTEBRATES

Technique	Sampling Device	Habitat	Replicates Composited for Wadeable Sites	Replicates Composited for Headwater Sites
Semi-Quantitaive				
$1m^2$ kicknet / seine	Kicknet / seine and wash bucket	Riffle	4 x $0.25m^2$	4 x $0.25m^2$
Multi-Habitat Sweep				
Undercut banks / roots	D-frame or triangular dip net and wash bucket	All applicable	3	3
Sticks / Wood			N/A	3
Emergent vegetation			3	N/A
Bedrock / slabrock			3	N/A
J. americana beds			3	N/A
Leaf packs		Riffle – Run – Pool	3	3
Silt, sand, fine gravel	US #10 Sieve	Margins	3	3
Aufwuchs sample	300 µm nitrex sampler / mesh	Riffle – Run - Pool	3	N/A
Rock pick	Fine-tipped forceps and wash bucket		15 total (5 each)	5 small boulders
Wood sample			3 to 6 linear meters	2 linear meters

After sampling has been completed, all sampling gear will be thoroughly cleaned to remove all benthic macroinvertebrates so that specimens are not carried to the next site. The equipment shall be examined prior to sampling at the next site to ensure that no benthic macroinvertebrates are present.

Habitat assessments will be performed at each of the macroinvertebrates sites. The habitat assessment method is covered in Section B2.2.4.

B2.2.3.1. Semi-Quantitative

In both headwater and wadeable streams, semi-quantitative sampling will consist of taking four (4) 0.25m^2 quadrat kick net samples from mid-riffle or the thalweg. This will be accomplished using a 0.25 m^2, 600µm mesh kick net, dislodging benthos by vigorously disturbing the 0.25 m^2 (20 x 20 in.) of substrate in front of the net. Large rocks will be hand washed into the net. The contents of the net will then be washed, and all four samples will be composited to yield a 1m^2 semi-quantitative sample. The composited sample will be partially field processed using a U.S. No. 30 sieve (600µm) and wash bucket. Large stones, leaves and sticks will be individually rinsed and inspected for organisms and then discarded. Small stones and sediment will be removed by elutriation using the wash bucket and U.S. No. 30 sieve. This sample must be kept separate from all other sub-habitat collections.

For headwater sites, two kick net samples will be allocated to each of two distinct riffles (at minimum) that are separated by at least one pool or run. This will be done to help reduce between-riffle variability. However, if there are several riffles located within the reach, the sampling effort will be spread across the reach to give a comprehensive evaluation of the entire community.

B2.2.3.2. Multi-Habitat

This method involves sampling a variety of non-riffle habitats with the aid of an 800 x 900µm mesh triangular or D-frame dip net. The habitats sampled and the number or size of replicates will differ for headwater and wadeable sites, as shown in Table 11. Each of these sub-habitat samples will be composited into one multi-habitat sample for each site.

Undercut Banks/Root Mats

These will be sampled by placing a large root wad into a triangular or D-frame dip net and shaking vigorously. The contents will be removed from the dip net and placed into a mesh wash bucket. If undercut banks are present in both run and pool areas, each will be sampled separately with three (3) replicates for both headwater and wadeable streams.

Marginal Emergent Vegetation (exclusive of Justicia americana beds)

This habitat will be sampled by thrusting (*i.e.,* "jabbing") the dip net into the vegetation for approximately 1m, and then sweeping through the area to collect dislodged organisms. Material will then be rinsed in the wash bucket, and any sticks, leaves and vegetation will be thoroughly washed and inspected before discarding. Three replicates will be conducted. This sub-habitat must be sampled for wadeable sites and may be sampled for headwater if present.

Bedrock or Slab-Rock Habitats

These habitats will be sampled by placing the edge of the dip net flush on the substrate, and disturbing approximately 0.1m^2 of area to dislodge attached organisms. Material will be emptied into a wash bucket, rinsed, inspected for organisms, and discarded. Three replicates will be conducted. This sub-habitat must be sampled for wadeable sites and may be sampled for headwater if present.

Justicia americana (water willow) Beds
These will be sampled by working the net through a 1m section in a jabbing motion. The material will then be emptied into a wash bucket, and any *J. americana* stems will be thoroughly washed, inspected and discarded. Three replicates will be conducted. This sub-habitat must be sampled for wadeable sites and may be sampled for headwater if present.

Leaf Packs
Leaf packs will preferably be collected from "conditioned" (*i.e.,* not new-fall material) material when possible. Samples will be taken from a diversity of habitats (*i.e.,* riffles, runs and pools) and placed into the wash bucket. The material will be thoroughly rinsed to dislodge organisms, inspected and discarded. Three replicates will be conducted for both headwater and wadeable sites.

Silt, Sand, and Fine Gravel
A U.S. No. 10 sieve will be used to sort larger invertebrates (*e.g.,* mussels, burrowing mayflies, dragonfly larvae) from silt, sand and fine gravel by scooping the substrate to an approximate depth of 5cm. A variety of collection sites will be sampled in order to obtain three (3) replicates in each substrate type where available (silt, sand and fine gravel). This sub-habitat will be sampled for both headwater and wadeable sites.

Aufwuchs Sample
Small invertebrates associated with this habitat will be obtained by washing a small amount of rocks, sticks, leaves, filamentous algae and moss into a medium-sized bucket half filled with water. The material will then be elutriated and sieved with the nitrex sampler/mesh. Three replicates will be conducted. This sub-habitat will be sampled only for wadeable sites.

Rock Picking
Benthic macroinvertebrates will be picked from 15 rocks (large cobble/small boulders; 5 each from riffle, run and pool) in wadeable streams and 5 small boulders from pools in headwater streams. Selected rocks will be washed in a bucket half filled with water and then carefully inspected to remove organisms.

Wood Sample
For wadeable streams, pieces of submerged wood, ranging from roughly 3 to 6 meters (10 to 20 linear feet) and ranging from 5–15 cm (2–6 inches) in diameter, will be individually rinsed into the wash bucket. For headwater streams only 2 linear meters will be sampled. Pieces of wood will be inspected for burrowers and crevice dwellers and will be removed with fine-tipped forceps. Large diameter, well-aged logs will be inspected and handpicked with fine-tipped forceps.

B2.2.3.3. Macroinvertebrate Identification and Analysis

Macroinvertebrate samples shall be delivered to Third Rock for identification according to *Laboratory Procedures for Macroinvertebrate Processing and Taxonomic Identification and Reporting* (KDOW. 2009b). After identification, macroinvertebrate sampling results

will be evaluated through calculation of several community metrics prescribed by KDOW 2008. Community metrics include taxa richness, EPT (mayfly, stonefly and caddisfly) richness, total number of individuals, modified percent EPT individuals, modified Hilsenhoff biotic index (mHBI), percent Ephemeroptera, percent primary clingers, and percent Chironomidae plus Oligochaeta (aquatic worms). Results of community metrics at each station will be combined to compute a Macroinvertebrate Bioassessment Index (MBI) score, ranging from 0 (worst) to 100 (best). MBI scores will be compared to scoring criteria developed by KDOW to arrive at water quality ratings of Very Poor, Poor, Fair, Good, or Excellent. For wadeable streams (watersheds greater than 5 mi^2) of the Bluegrass Bioregion, a MBI score below 20 is Very Poor, from 21 to 40 is Poor, from 41 to 60 is Fair, from 61 to 79 is Good, and greater than 70 is Excellent. For headwater streams (watersheds less than 5 mi^2) of the Bluegrass Bioregion, a MBI score below 18 is Very Poor, from 19 to 38 is Poor, from 39 to 50 is Fair, from 51 to 57 is Good, and greater than 58 is Excellent (KDOW 2008).

Results from this project will be compared with Bluegrass Bioregion Criteria, reference reach scores, and results from MS4 permit sampling at the mouth of Wolf Run. These results and the results of the watershed habitat assessment monitoring will be combined into a final report entitled "Assessment of Habitat and Macroinvertebrates in the Wolf Run Watershed" due to the KDOW and LFUCG by December 31, 2011.

B2.2.4. Watershed Habitat Assessments

Habitat assessments will include a visual assessment of ten habitat parameters that characterize the stream "micro scale" habitat, the "macro scale" features, and the riparian and bank structure features that are most often influential in affecting the other parameters. The method follows the US EPA's *Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers* (Barbour *et al.* 1999). Each of the parameters will be evaluated on a "Condition Category" scale from 0 to 20. The categories within this scale include "Optimal" for scores from 20 to 16, "Suboptimal" for scores from 15 to 11, "Marginal" for scores from 10 to 6, and "Poor" for scores from 5 to 0. The score for each parameter will be summed to produce a final habitat score (maximum 200).

For parameters 1 to 5, the habitat assessment will evaluate a composite of the entire biological sampling reach. For parameters 6 to 10, an area beginning approximately 100-m upstream of the sampling reach through the sampling reach will be evaluated as a composite. The evaluator will face downstream when determining left and right bank. For parameters 8 to 10, each bank will be scored independently from 10 to 0. At each sampling site, results will be recorded on the High-Gradient Habitat Assessment Field Data Sheet. Photographs will be taken to document upstream and downstream conditions.

The following paragraphs summarize each of the ten parameters assessed.

Parameter #1 - Epifaunal Substrate/Available Cover

This metric measures the relative quantity and the variety of stable structures, such as cobble, boulders, fallen trees, logs, branches, root mats, undercut banks, aquatic

vegetation, etc., that provide refugia, feeding opportunities and sites for spawning and nursery functions.

Optimal: >70% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at a stage to allow full colonization potential (*i.e.,* logs/snags that are not new fall and not transient).

Suboptimal: 40%-70% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of new fall, but not yet prepared for colonization (may rate at the high end of the scale).

Marginal: 20%-40% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.

Poor: <20% stable habitat; lack of habitat is obvious; substrate unstable or lacking.

Parameter #2 Embeddedness

Embeddedness describes the extent to which rocks and snags are covered or sunken into the silt, sand, mud or biofilms (algal, fungal or bacterial mats) of the stream bottom. Generally, as rocks become embedded, the surface area available to macroinvertebrates and fish (for shelter, spawning and egg incubation) is decreased; assess in the upstream or central portions of riffles.

Optimal: Rocks are 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space.

Suboptimal: Rocks are 25%-50% surrounded by fine sediment.

Marginal: Rocks are 50%-75% surrounded by fine sediment.

Poor: Rocks are >75% surrounded by fine sediment.

Parameter #3 - Velocity/Depth Regime

The best streams in most high-gradient regions will have all of the following patterns of velocity and depth: 1) slow-deep, 2) slow-shallow, 3) fast-deep and 4) fast-shallow; the occurrence of these four patterns relates to the stream's ability to provide and maintain a stable aquatic environment. Investigators may have to scale deep and shallow depending upon the stream size; a general guideline is 0.5 m between shallow and deep.

Optimal: All 4 regimes present.

Suboptimal: Only 3 of the 4 regimes present; if fast-shallow is missing, score lower than if missing other regimes.

Marginal: Only 2 of the 4 regimes present; if fast-shallow or slow-shallow are missing, score low.

Poor: Dominated by 1 regime (usually slow-deep).

Parameter #4 - Sediment Deposition

This metric measures the amount of sediment that has accumulated in pools and changes that have occurred to the stream bottom as a result of deposition. This may cause the

formation of islands, point bars (areas of increased deposition usually at the beginning of a meander that increases in size as the channel is diverted toward the outer bank) or shoals or result in the filling of runs and pools. Sediment is often found in areas that are obstructed and areas where the stream flow decreases, such as bends. Deposition is a symptom of an unstable and continually changing environment that becomes unsuitable for many organisms. Examine bars/shoals and pool substrates within the biological monitoring station, when assessing this parameter.

Optimal: Little or no enlargement of islands or point bars and less than 5%of the bottom affected by sediment deposition.

Suboptimal: Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5%-30% of the bottom affected; slight deposition in pools.

Marginal: Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30%-50% of the bottom affected; moderate sediment deposits apparent at most obstructions and slow areas, bends and pools.

Poor: Heavy deposits of fine material; increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.

Parameter #5 - Channel Flow Status

This metric measures the degree to which the channel is filled with water. The score will change with the seasons. Estimate the percentage of the channel that is wet using the low water mark.

Optimal: Water reaches base of both lower banks; minimal amount of channel substrate exposed.

Suboptimal: Water fills >75% of the available channel or <25% of channel substrate exposed.

Marginal: Water fills 25%-75% of the available channel; riffle substrates are mostly exposed.

Poor: Very little water in channel; mostly present in pools.

Parameter #6 - Channel Alteration (Both Sheets)

This metric measures the large-scale, direct changes in the shape of the stream channel. Channel alteration is present when 1) artificial embankments, rip-rap and other forms of bank stabilization or structures are present, 2) the stream is very straight for significant distances because of channelization, 3) dams and bridges are present that obstruct flow and/or 4) dredging or other substrate mining activities are occurring or have occurred.

Optimal: Channelization or dredging absent or minimal; stream with normal pattern.

Suboptimal: Some channelization present, usually in areas of bridge abutments; evidence of past channelization (dredging, etc., >20 past years) may be present, but recent channelization not present.

Marginal: Channelization may be extensive; embankments or shoring structures present on both banks; and 40%-80% of the stream reach channelized and disrupted.

Poor: Banks shored with gabion or cement; >80% of the stream disrupted; in stream habitat greatly altered or removed entirely.

Parameter #7 - Frequency of Riffles (or Bends)

This metric measures the sequence of riffles and thus the heterogeneity occurring in a stream. Estimate riffle frequency by determining the ratio of distance between riffles divided by the width of the stream. An average of the riffle ratios is determined for biological monitoring stations and the upstream segment.

Optimal: Occurrence of riffles relatively frequent; ratio of distance between riffles divided by the width of the stream <7:1 (generally 5 to 7); variety of habitat is key; in streams where riffles are continuous, placement of boulders or other large, natural obstruction is important.

Suboptimal: Occurrence of riffles infrequent; distance between riffles divided by the width of the stream is between 7 and 15.

Marginal: Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 and 25.

Poor: Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is >25.

Parameter #8 - Bank Stability

This metric measures whether the stream banks are eroded or have the potential to erode. Each bank is scored independently from 10-0.

Optimal: Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems; <5% of bank affected.

Suboptimal: Moderately stable; infrequent, small areas of erosion mostly healed over; 5%-30% of the bank affected.

Marginal: Moderately unstable; 30%-60% of bank in reach has areas of erosion; high erosion potential during floods.

Poor: Unstable; many raw, eroded areas; obvious bank sloughing; >60% of bank has erosional scars.

Parameter #9 - Bank Vegetative Protection

This metric measures the amount of vegetative protection afforded to the stream and the nearstream portion of the riparian zone. Each bank is scored independently from 10-0. Determine what vegetative types (trees, understory shrubs, herbs and non-woody macrophysics) are present on each bank. Those stream banks with different vegetative types provide better erosion protection and provide more of a variety of allochthonous food material. Native vegetation scores higher than invasive or non-native vegetation.

Optimal: >90% of the stream bank surfaces and immediate riparian zones covered by natural vegetation, including trees, understory shrubs, herbs and non-woody macrophytes; vegetation disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.

Suboptimal: 70%-90% of the stream bank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one half of the potential plant stubble height remaining.

Marginal: 50%-70% of the stream bank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one half of the potential plant stubble height remaining.

Poor: <50% of the stream bank surfaces covered by vegetation; disruption is very high; vegetation has been removed to 5 cm or less in average stubble height.

Parameter #10 - Riparian Vegetative Zone Width

This metric measures the width of the natural vegetation from the edge of the stream bank through the riparian zone. The presence of old fields, paths, walkways, etc., in otherwise undisturbed riparian zones may be judged to be inconsequential to highly destructive to the riparian zone. Each bank is scored independently from 10-0. When determining final scores, the age and density of the riparian vegetation should be evaluated (*e.g.,* A score of 9, instead of 10, should be given to a riparian zone that is over 20 m in width, but is dominated by 5-10 year old hardwood trees).

Optimal: Width of riparian zone >18 m; human activities (parking lots, roadbeds, clear-cuts, lawns, pastures or crops) have not impacted the zone.

Suboptimal: Width of riparian zone 13-18 m; human activities have impacted the zone only minimally.

Marginal: Width of riparian zone 6-12 m; human activities have impacted the zone a great deal.

Poor: Width of riparian zone <6 m; little or no riparian zone due to human activities.

All habitat assessments will be completed by October 31, 2011 and submitted to the FOWR Sampling Coordinator. The FOWR Sampling Coordinator will compile these results into an electronic Microsoft Excel database. The database, along with electronic copies of the field datasheets will be submitted to Third Rock by November 30, 2011 for incorporation into the final report discussing the results of the habitat assessments and the macroinvertebrate survey.

B2.2.5. Hydrogeomorphic Assessment

Three types of measurements will be made in the hydrogeomorphic assessment: cross-sections, longitudinal profiles, and pebble counts. These measurements will be made at each of the nine hydrogeomorphic monitoring sites. Permanent monuments consisting of rebar (0.75-inch rebar or similar material approximately 4 feet long) concreted within a plastic pipe casing shall be installed at the permanent cross-section survey sites. A monument shall be installed on both the right and left stream banks at least 10 ft back

from the top of bank, indicating the extent of the measured cross-section and can serve as surveying benchmarks. If installation of such monuments is not feasible, other permanent monuments will be established. To facilitate profile relocation during the second surveying period, the following actions shall be taken in the field:

1) monuments shall be marked with a piece of flagging or paint,
2) GPS points shall be recorded at monuments and any other locations that would aid in site relocation,
3) photographs will be taken (for both relocation and to document the current site conditions), and
4) notes will be recorded on site identification characteristics (e.g. bank condition, distinguishing landmarks/features, and other pertinent data).

The methods for each of these sampling efforts are described below.

B2.2.5.1. Cross-Sections

Cross-sections to be surveyed will be located within riffle features and identified by permanent monuments. Points will be taken frequently at horizontal stations within each cross-section such that the surveying indicates all significant breaks in slope and provides a thorough characterization of each cross-section (refer to USFS, 1994 for surveying procedures). Equipment used will include a 50- or 100-ft surveying tape, laser level (leveling accuracy < +/- 3mm/30m) on a tripod, and surveying rod. Data may be recorded in RiverMorph™ software using a Rugged Reader Pocket PC or in a field notebook. Surveying precision shall be +/- 0.01 ft for vertical readings and +/- 0.1 ft for horizontal readings. Notes related to observed changes at various elevations within the cross-section will be made. Each stream permanent cross-section will be surveyed twice, once at the initial site visit following monument installation and approximately nine months subsequent to first measurement. Differences between these two measurements will allow estimation of channel change and if degradation is occurring, the erosion rate can be calculated.

B2.2.5.2. Profiles

Representative stream longitudinal profiles will be taken over a distance that includes approximately three riffle features at each of the nine hydrogeomorphic monitoring stations. Permanent monuments on a designated bank and at least 10 feet back from the top of bank will mark the upstream and downstream extents of the profiles and can serve as benchmarks for surveying. Profile measurements will be taken within the stream thalweg and will be of adequate frequency to identify all grade changes and facet slopes within the profile (refer to USFS, 1994 for surveying procedures). Equipment used will include a 100-ft surveying tape, laser level (leveling accuracy < +/- 3mm/30m) on a tripod, and surveying rod. Data will be recorded in RiverMorph™ software using a Rugged Reader Pocket PC or in a field notebook. Surveying precision shall be +/- 0.01 ft for vertical readings and +/- 0.1 ft for horizontal readings. Locations of permanent cross-sections and pebble count monitoring will be indicated within the recorded profile. Each stream profile will be surveyed twice, once at the initial site visit following monument installation and approximately nine months subsequent to first measurement. Differences

between these two measurements will allow estimation of changes to channel bed elevation, facet slope, and facet length.

B2.2.5.3. Pebble Counts

Reach-wide pebble counts will be collected within the stream where the longitudinal profiles are taken at the nine hydrogeomorphic monitoring sites. If substrate does not appear similar in all riffles, riffles with considerably coarser substrate that could be indicative of a large rock fall will be avoided. Each reach pebble count will sample within the riffles and pools proportional to the length of the reach comprised of riffles and pools. Riffle and pool data will be kept separate, but can be combined later to produce a reach average particle distribution. For the reach-wide pebble counts, particle sampling will be completed along evenly spaced transects over the entire bankfull width and consist of at least 100 particles (refer to Rosgen, 2008 and Bunte and Steven, 2001 for pebble count procedures). Since much of the bed material in these streams is predominantly sand, silt, and clay or bedrock a higher number of particles sampled is not indicated. If it is determined upon field investigation that a given hydrogeomorphic monitoring site has a wide particle-size spectrum, at least 400 particles may be collected for the reach-wide pebble count.

An active bed, riffle pebble count will also be collected within the permanent cross-section at each of the nine hydrogeomorphic monitoring sites. For the active riffle bed count, particle sampling will be completed along evenly spaced transects over the active bed width and consist of at least 100 particles (refer to Rosgen, 2008 and Bunte and Steven, 2001 for pebble count procedures).

For all pebble counts, each transect will start on the same side of the stream and collection will move from downstream to upstream. Sampling points will be spaced by at least the D_{max} particle size. The pebble count will end at the extent of a given transect, not in an arbitrary location when a count of 100 particles is reached. If fine sediments (sand/silt) are encountered and the thickness of the sediment layer is less than 0.5 inch, then it will be appropriate to select the larger particle below the fines. Otherwise the observation will be counted as fines (*i.e.* less than or equal to 2mm). Equipment used will include a ruler (mm) or gravelometer (gravel template), with the gravelometer being preferred. Data may be recorded in RiverMorph™ software using a Rugged Reader Pocket PC or on a Pebble Count Datasheet (see Appendix A). Precision for pebble count readings will be such that each data point measures within +/- 1 units of the narrative particle description or +/- 0.5 phi units on the gravelometer. Each pebble count will be performed twice, once at the initial site visit and approximately nine months subsequent to first measurement. For each sampling event, particle size distributions and D_{50} values will be computed and differences between these two measurements will allow estimation of changes to channel substrate.

B2.2.6. Water Quality Monitoring

Water quality monitoring will be conducted at ten (10) monthly sampling events at a minimum of twelve (12) sampling stations in the watershed during dry and wet conditions. The sampling date within each month will be flexible such that at least two

of the events will be considered 'wet-weather' and two of the events will be considered 'dry-weather.' The FOWR Sampling Coordinator shall work with the Third Rock Sampling Coordinator to schedule sampling dates each month. A minimum notice of 48 hours is required prior to mobilization of the volunteer samplers.

During the monthly sampling, grab samples will be collected by volunteers and delivered within six hours of collection to the LFUCG Town Branch laboratory for analysis of *E. coli*, fecal coliform, total suspended solids, ammonia, nitrite, total dissolved solids, alkalinity, and hardness. Samples for total phosphorus, ortho-phosphorus, total kjeldahl nitrogen, and nitrate will be delivered to the Kentucky Geological Survey laboratory. Volunteers will perform field measurements of turbidity, dissolved oxygen, specific conductance, temperature, and pH. Third Rock will accompany the volunteers during each event to conduct discharge monitoring and collect the field filtered ortho-phosphorus sample. Also due to the short time period in which wet-weather events can be collected on the hydrographic rise, two wet-weather sampling events will be collected solely by Third Rock staff. If for some reason, volunteers are not able to perform the sampling, Third Rock will collect additional sampling events. Efforts will be made to avoid collecting samples on Thursday, as this is the busiest day at the Town Branch laboratory.

In addition to the monthly sampling, volunteers will collect an additional four events for *E. coli* and fecal coliform to evaluate the geometric mean for the primary contact period. A Third Rock staff member will accompany the volunteers during each event to conduct discharge monitoring. Only flow and *E. coli* and fecal coliform will be collected during these events. The LFUCG Town Branch laboratory will analyze the samples.

Subsequent to the completion of all sampling, results will be compiled and analyzed in a final "Wolf Run Watershed Monitoring Report." Further, this data will be used in conjunction with other data collection efforts in the watershed to produce the loading calculations for Chapter 4 of the Wolf Run Watershed Based Plan.

The methods to be utilized in performing these tasks are listed in the sections below.

B2.2.6.1. Flow Measurement

The procedure for flow measurement is explained in B2.2.1. Flow measurements will be conducted according to the KDOW's *Measuring Stream Discharge Standard Operating Procedure* (KDOW 2010b).

Velocity and flow area measurements must manually be recorded in a bound field book, or on other appropriate field data sheets, using indelible, waterproof ink and waterproof paper. Discharge values will be calculated in the office according to the equations specified in KDOW 2010b. One duplicate measurement will be recorded per sampling event.

B2.2.6.2. Grab Sampling

Grab sampling will be conducted according to the Kentucky Division of Water's *Sampling the Surface Water Quality in Lotic Systems* (KDOW 2011). The methods specific to this collection effort are described below. One field duplicate sample will be collected for all parameters per sampling event.

Samplers should put on powderless latex or nitrile gloves prior to sampling. Grab samples should be collected in the centroid of flow in a section of stream in which indicators of complete mixing are evident. The sampler should face upstream and approach the site from downstream, ensuring that no disturbed streambed sediment contaminates the sample. If additional work is planned upstream of the sample site, the water samples must be taken first. Care should be taken not to displace the preservative since sample bottles are pre-prepared.

If bottles are not pre-preserved, triple rinse the sample bottle with stream water prior to sample collection. When sampling, point the mouth of sample container upstream/against the flow. Submerge the entire bottle and fill it with water. If the stream is too shallow to fill the bottle while submerged, fill as much as possible while submerged, ensuring the minimal amount for analysis is obtained. Also if the bottle contains a preservative, angle the mouth so as not to spill the preservative while collecting. Rinse the caps with sample water prior to capping the bottle. Samples should be stored in containers that are free of potential contaminants. Sample bottles may be placed inside sealed food-grade plastic bags prior to being stored on ice in coolers to improve laboratory sorting and reduce potential cross-contamination.

For bacterial samples, fill bottles to the inscribed 'fill line'. Do not pour off excess water. If the bottle is filled above the 100ml fill line, the excess will be decanted during laboratory analysis. If adequate volume is not obtained on first effort, do not reuse the bottle. Use a new, un-contaminated bottle and repeat the procedure. Close and secure the sample bottle lid immediately and preserve sample accordingly.

B2.2.6.3. Field Filtration

The collection of the ortho-phosphorus samples requires field filtration using a hand pump. Third Rock will perform this field filtration within 15 minutes of sample collection. In order to collect this field filtered sample, the stream sample will be collected using the grab sample methodology. The funnel, funnel filter base and flask will be triple rinsed with with DI water, and the hand pump, the inside of tubing and tweezers will be single rinsed with DI water. Clean forceps will be used to place 0.45 µm paper filter onto funnel filter base. The filter base will be attached to flask and the tubing from the hand pump will be connected. 50 mL DI water will be poured into funnel, filtered, rinsed and discarded. 50 mL of the stream sample water will be poured into funnel, filtered, rinsed, and discarded. Then enough stream sample water will be poured into the funnel to provide enough finished sample for rinsing the storage bottle and for analysis. If the stream is particularly turbid, smaller amounts of the sample water will be used. When 0.45 µm paper filter becomes excessively clogged, the filter will be removed with forceps, discarded, and replaced with a fresh filter. Filtering will be continued until the

required sample volume is achieved. The sample will be poured from the filter flask into the sample bottle.

B2.2.6.4. *In-situ* Measurements

Volunteers will perform field measurements of turbidity, dissolved oxygen, specific conductance, temperature, and pH. The sampling methods for temperature and pH are specified in the Watershed Watch Water Chemistry Sampling Methods for Field Chemistry and Lab Analysis (Appendix B). The temperature must be recorded to an accuracy of 0.5 °C and pH to an accuracy of 0.5 SU. Instructions for the dissolved oxygen and specific conductivity methods are specified in the instruction manuals associated with the meter / test kits (see Appendix B). Turbidity will be evaluated through a visual assessment and indicated as "clear", "slightly turbid", "turbid", or "other". Results will be recorded on the chain-of-custody (Appendix A).

During the two wet-weather sampling events collected by Third Rock, Hydrolab multimeters will be used to record the dissolved oxygen, specific conductance, temperature, and pH. The procedures specified in *In-situ* Water Quality Measurements and Meter Calibration Standard Operating Procedure (KDOW, 2009a) will be used in these measurements. Results will be recorded in the field notebook.

At one site per sampling event, replicate measurements will be made on all in-situ parameters except turbidity.

B3. Sample Handling and Custody Requirements

The sample handling and custody procedures are compatible with the SOP "Sample Control and Management" (KDOW 2009c).

B3.1. Chain-of-Custody

Chain-of-Custody (COC) forms will be completed for all samples collected in the field and will follow each sample throughout sample processing. A COC form is a controlled document used to record sample information and ensure the traceability of sample handling and possession is maintained from the time of collection through analysis and final disposition. A sample is considered in custody if it is:

- In the individual's physical possession
- In the individual's sight
- Secured in a tamper-proof way by that individual, or secured in an area restricted to authorized personnel

Example COCs that will be used in the collection are attached in Appendix A. All information shall be documented on the COC in black or blue waterproof permanent ink including field physical measurements and custody information.

The sampler shall initiate sample custody at the time the sample is collected. Field custody documentation shall include:

- Verification of sample identification
- Number of sample bottles collected
- Collection date
- Collection time
- Collector's signature

The sampler shall maintain possession of the sample until custody is transferred to the laboratory or another party. The COC shall accompany the sample from the time of collection until it is relinquished. Field custody will be relinquished by signature, with date and time, of the sample in the designated area on the COC.

B3.2. Sample Handling and Transport

The sampler will be responsible for sample handling in the field and transport of samples to the laboratory. The sampler will collect the sample at the source following established protocols. The sampler will be responsible for collecting the sample in appropriately identified collection containers with the correct preservative, as applicable, and ensuring that the container lid is secured tightly to prevent leakage or outside contamination. Sample containers for chemical analysis shall be immediately placed in a cooler on ice to maintain a temperature of 4±2° C for transport to the laboratory. Sample bottles shall be placed in the cooler with lid side up in an organized manner per COC entry.

Sample coolers will be of adequate size to allow ice to surround all sample bottles. It is the responsibility of the sampler to ensure that coolers are properly packed in the field and that they have sufficient cooler space on their vehicle for their daily sample load. Coolers shall be secured during transport such that significant disturbance of the samples is avoided. Macroinvertebrate samples will be analyzed at Third Rock. The LFUCG Town Branch laboratory and Kentucky Geological Survey laboratory will process the chemical sampling parameters.

Upon receipt at the laboratory, the sample custodian shall review the COC for completeness and accuracy. Anomalies shall be documented. The laboratory shall measure sample temperature upon receipt, determine if sample aliquots have been placed in appropriate bottles and properly preserved, and inspect the sample for proper identification and bottle integrity; any discrepancies and/or bottle damage shall be documented on the COC. If the hold time requirement is exceeded for any parameter, the result is qualified and a re-sampling must be scheduled.

B3.3. Sample Labeling

Whenever possible during field preparation while in the office, sample bottles will be labeled to prevent information omission. Bottles can be labeled in the field, as long as the following minimum requirements for labeling are followed. All bottles must have the following information recorded either on a sample tag or label affixed to the container, or written directly on the container:

- Sample identification (unique site ID number)
- Date of collection

- Time of collection (formatted in military time, or indicate am or pm)
- Type of analysis requested
- Type of sample (grab, composite, semi-quantitative, multi-habitat)
- Media (surface water, biological-macroinvertebrates)
- Preservative (ice, acidification, etc.)
- Collector's initials

For macroinvertebrate samples, the stream name and location will also be documented on the label.

B3.4. Sample Designation

Sampling technicians will be responsible for recording the unique sample identification, as well as the date and time of the collection on each sample bottle. The unique sampling event code will follow the following format:

SAMPLE ID = W##-YYMMDD

Where:

W## is Unique Site Identifier (0-12 or "DD" for Duplicate)
YYMMDD is the date in year (YY), month (MM), day (DD) format.

As indicated above, duplicate samples will be indicated as such in the site identifier of "WDD." The time of collection will not be indicated on the chain-of-custody so that the laboratory is blind as to the sampling location it corresponds with. This information shall be recorded in the field notebook during collection so that results can be compared after analysis.

B4. Analytical Methods Requirements

USEPA methodology must be used for all analysis as applicable. Detection limits for all parameters must be at a sensitivity level to compare to Kentucky water quality standards. The requirements for all methods and detection limits are specified in Table 6. SOPs for the chemical laboratory methods are specified in Price 2009.

Third Rock's macroinvertebrate identification laboratory will follow laboratory protocols for benthic macroinvertebrate sample processing, identification and data reporting per KDOW (2009b, 2008) with the following exceptions:

- All samples will be logged into Third Rock's Macroinvertebrate Laboratory Information Management System (MacLIMS) upon receipt.
- Sample identification date will be maintained in MacLIMS.
- Taxonomic QA/QC dates (if applicable) will be noted on individual QA/QC forms and maintained electronically in the Project File.
- Initials of the applicable party completing each task associated with sorting, identification, or quality control will be noted electronically in MacLIMS or on associated QA/QC forms.

- QA checks will be documented on applicable forms and maintained in associated project files. These forms include the Macroinvertebrate Sample Sorting Efficiency Form, Macroinvertebrate Sample Taxonomy Precision Form, and Macroinvertebrate Sample Taxonomic and Enumeration Efficiency Form.

B5. Quality Control Requirements

B5.1. Field Quality Controls

Field quality control checks for water chemistry will be collected at a frequency of one duplicate every sampling event. Field duplicates must be randomly determined from the 12 sites and recorded on field datasheets or project logbooks. The field controls shall be performed as follows.

A random number table (see Appendix A) will be used to select one sampling site such that all sample parameters are duplicated. Two separate samples will be collected for each parameter. The samples will be collected at the same time and at the same location. One sample will be labeled as usual, and the other sample will have the site name indicated as a "duplicate." On a form separate from the COC, the site from which the duplicates were collected will be documented. In addition to the duplicate grab sample collection, duplicate in-situ measurements and flow measurements will also be made at this site.

Duplicate habitat assessments will be performed by all volunteer samplers as well as by Third Rock biologists at the six macroinvertebrate sampling sites. These duplicate measurements will be used to evaluate precision amongst volunteers conducting habitat assessments.

During the karst characterization, a minimum of five percent of measurements will be duplicated.

B5.2. Macroinvertebrate Taxonomic Quality Controls

Ten percent of all sorting pans will be checked by a second sorter to assure that samples have been picked thoroughly. These samples will be selected randomly using the MacLIMS database programming. This check is documented on the Taxonomic & Enumeration Efficiency Form.

Five percent of all identified samples will be re-identified to insure QA/QC by a second taxonomist. These samples will be selected randomly using the MacLIMS database programming. Ninety percent or greater composition comparability (*e.g.,* abundance and richness) is the target success criteria. If there is less than 90 percent comparability between the taxonomists, then taxonomy must be reconciled by both taxonomists and a third taxonomist, if deemed necessary. This quality control process shall be documented on the Macroinvertebrate Sample Taxonomy Precision Form and Macroinvertebrate Sample Taxonomic and Enumeration Efficiency Form and included in the monitoring report.

B5.3. Chemical Laboratory Quality Controls

Laboratory quality controls will be analyzed as specified in the SOPs listed in *Quality Assurance Plan (QAP) and Standard Operating Procedures (SOPs)* (Price 2009). These controls include method blanks, matrix spikes, calibration check samples, laboratory replicates, and other method specified controls. The frequencies of analysis for these standards are all specified by the individual methods.

B6. Instrument / Equipment Testing, Inspection, Calibration, and Maintenance Requirements

Laboratory instrumentation will be maintained according to the SOPs listed in Table 5. Field sampling equipment will be maintained as specified in KDOW 2009b and Table 12. The record of inspection, calibration, and maintenance will be recorded in a instrument logbook maintained by the sampler. For sampling nets and bottles, inspection will ensure that the items are free from contamination, in good condition, and adequate for use.

TABLE 12 – FIELD EQUIPMENT CALIBRATION AND MAINTENANCE

Equipment Name/Type	Equipment Purpose	Inspection Frequency	Type of Inspection	Calibration Frequency	Standard or Calibration Instrument Used	Person Responsible
Multimeter for Temperature, pH, Dissolved Oxygen and Conductivity	Physicochemical Monitoring	Before each collection event	Overall condition/ battery power	Before each field sampling event	Calibration standards, user manual	Sampler
EC Conductivity PockeTesters	Conductivity Measurement	Before each collection event	Overall condition/ battery power	Before and after each uses	Calibration standards, user manual	Sampler
Hand pumps, filter funnel, tubing, and flasks	Field Filtration	Before each collection event	3 solution wash, overall condition	N/A	N/A	Sampler
Flow Meter	Discharge Monitoring	Before each collection event	Overall condition/ battery power	Annual	Manufacturer	Sampler
TROLL® Dataloggers	Karst Characterization Stage Measurement	Before use	Cleanliness, program settings	N/A	N/A	Sampler
Macroinvertebrate Sampling Nets	Macroinvertebrate Sampling	Before each use	Overall condition	N/A	N/A	Sampler
Sample Bottles	Sample Collection	Before collection	Good condition	N/A	N/A	Sampler

Volunteer samplers will calibrate the conductivity meters before and after each sampling event. Two standards will be used to calibrate the meters with results recorded on the chain-of-custody or Conductivity Survey Field Datasheet (Appendix A).

Third Rock calibrates flow meters annually through the manufacturer. The multimeters to be used will be calibrated according to manufacturers instructions prior to each

sampling event using a three point pH calibration and a one point conductivity calibration. All results will be recorded in the instrument logbook.

The filter funnel, tubing, and flask used in field filtration will be cleaned prior to sampling or weekly at maximum. To clean the equipment, three clear HDPE washbasins will be used. One washbasin will be labeled "Detergent Wash", one "Acid Solution" and the final "DIW." The detergent used for cleaning equipment must be certified phosphate-free. All washbasins used during the cleaning process must be pre-cleaned following the same procedures:

1. Detergent Wash and Tap Water Rinse
   a. Put on powderless nitrile gloves
   b. Place equipment in basin labeled "Detergent Wash" and soak equipment in a tap water/detergent mix for 30 minutes
   c. Fill tubing with solution and keep submerged for 30 minutes
   d. Scrub exterior and interior surfaces of equipment
   e. Rinse thoroughly with warm tap water to remove detergent residue
2. Acid Soak and Rinse
   a. Put on a new pair of gloves
   b. Place equipment and tubing into a washbasin labeled "Acid Solution"; for pieces of equipment that contain metal parts, skip to Step 3.
   c. Fill washbasin with 5% HCl solution (ACS trace-element grade HCL; 5% by volume in DIW).
   d. Soak for 30 minutes; Stir solution occasionally to promote the detachment of organic and inorganic contamination from the equipment
3. DI Water Rinse
   a. Put on a new pair of gloves
   b. Place equipment and tubing into a washbasin labeled "DIW"
   c. Rinse all equipment and tubing with DI water
   d. Place onto a clean surface to dry
4. Clean Equipment Storage
   a. Place clean equipment in plastic storage bags
   b. Double bag tubing

B7. Data Management

In order to ensure that project objectives are achieved, data must be collected and managed in a manner that will protect and ensure its integrity. The data collected under this project will be produced under standardized procedures and forms, where practicable.

All field data will be recorded using black or blue indelible ink. Mistakes on field data sheets will be crossed out with one line (so the information is still discernible), with the initials and date of the person making the correction. The correct information will then be recorded legibly on another line, or above or below the original info. If a separate sheet is necessary for new information, the original sheet will be attached to the new sheet, and initialed and dated.

Data collected by volunteers shall be submitted to the Ken Cooke, FOWR Sampling Coordinator. He shall review the data for any nonconformances, and enter the data into electronic databases and forward the electronic databases and electronic copies of the original datasheets to the QA Manager. He will be responsible for storing all original copies of the volunteer field data collected.

Chemical laboratory analytical results and internal laboratory logbook documentation will be the responsibility of David Price, Laboratory Director. Macroinvertebrate laboratory results and metric calculations will be the responsibility of Bert Remley, Macroinvertebrate Laboratory Chief Taxonomist. Upon completion of laboratory analysis, results shall be forwarded electronically to Steve Evans, QA Manager.

Data collected by Third Rock samplers and staff will be maintained by Steve Evans, QA Manager. He will receive the laboratory reports and review the data for conformance to the requirements of this QAPP and will subsequently send the results to the KDOW and LFUCG. He will be responsible for sending all hardcopy and electronic copies of data reports to the LFUCG and KDOW, as well as maintaining previously submitted data. All final reports will receive a technical peer review and a grammatical / formatting review prior to submission.

All raw data, documentation, and records shall be retained. Correspondence and other documentation relating to interpretation and evaluation of data collected, analyzed, or processed shall also be retained. The retention period is a minimum of three years subsequent to grant completion. All data maintained on Third Rock computers will be supported by a daily backup and archival system. Hard copy files will be stored onsite under secure conditions.

No data shall be publicly disseminated unless first reviewed and approved for release by the Project Team including the Grantee Project Manager, QA Manager, and FOWR Sampling Coordinator. Prior to that time, data will be managed by Third Rock. Subsequent to final approval, Project Team members may distribute the results as appropriate.

SECTION C – ASSESSMENT AND OVERSIGHT

Assessment and response actions are necessary to ensure that this QAPP will be implemented as approved. For a general summary of these assessments see Table 13. If at any time a project team member finds an error or non-conformance in the QAPP, the QAPP will be revised and redistributed to those on the distribution list subsequent to approval. The KDOW QA officer may freely review all field and laboratory techniques as requested. Any identified problems will be corrected based on recommendations by the KDOW QA Officer.

TABLE 13 – WATERSHED ASSESSMENT AND MANAGEMENT REPORTS

Type	Frequency	Purpose	Parties Responsible For		Reporting Method
			Performing	Responding	
QAPP Revision	As necessary	Address non-conformances or errors in the QAPP	Project Team Members	QA Manager	Distribution of amended QAPP
KDOW Audit	As requested	Ensure conformance to project objectives	KDOW	Parties of concern	Corrective Action Response
Laboratory Demonstration of Performance	Prior to initial analysis	Ensure analyst is capable of performing the method to specifications.	Laboratory QA Director	Laboratory Analysts	Internal Lab documentation
Laboratory Internal Audits	Annually, at minimum	Ensure conformance to methods, regulations, and procedures.	Laboratory QA Director	Laboratory Analysts	Internal Lab documentation
Progress Assessment	Quarterly	Evaluate the status on project related objectives and concerns	Grantee Project Manager, or designee	KDOW	Section 319(h) Nonpoint Source Project Progress Report
Volunteer Field Sampling Audit	Once during project	Assess volunteer sampler conformance to proper sampling and documentation protocols.	QA Manager, or designee	Volunteer Samplers, Trainers	Field Sampling Audit Checklist
Analytical Results Review	Subsequent to each sampling event	Evaluate the conformance of laboratory data to project DQOs	QA Manager, or designee	Laboratory QA Director	Laboratory Data Quality Checklist
Quality Evaluation	Once, End of Project	Evaluate the quality assurance and compare the data produced to project DQIs	QA Manager, or designee	KDOW QA Officer, Grantee Project Manager	Final Monitoring Reports

To ensure conformance with this QAPP and the applicable regulations, certifications, and methods by which the laboratories operate, the laboratories will perform several assessment measures. To ensure that analysts are capable of performing the requested analytical methods to specifications, each analyst must acceptably demonstrate this ability prior to conducting sample analyses. The analyst must conduct four replicate analyses of a known standard and achieve precision and accuracy equal to or better than the acceptance ranges for laboratory duplicates and laboratory control samples, respectively. The laboratory QA Director or his appointee on an annual basis will perform internal audits. The findings of the audits, both positive and negative, will be documented, and the corrective response to the cited deviations will be made. Corrective actions will be submitted to the auditing body for review and approval.

The QA Manager is responsible for the overall conformance of field personnel to the procedures, protocols, and methods established by this QAPP and internal project related procedures. The QA Manager will conduct at least one audit of the volunteer samplers field activities and documentation including calibration and maintenance of field equipment and sample collection techniques. Deviations found in such assessments will

be reported to the samplers and documented using the Field Sampling Audit Checklist (Appendix A).

Upon receipt of the results, a review of the laboratory data shall be performed by the QA Manager or his designee to ensure that the project DQOs have been satisfied. The Laboratory Data Quality Checklist (see Appendix A) shall be utilized to document this review. The final reports for each of the monitoring activities will include an evaluation of the quality assurance and will compare the data produced under the water quality monitoring to the data quality indicators listed herein.

Quarterly Section 319(h) Nonpoint Source Project Progress Report will be submitted to the KDOW by the Grantee Project Manager to document the progress on the project milestones.

SECTION D – DATA VALIDATION AND USABILITY

D1. Data Review, Validation and Verification

Data review is the internal examination to check if data has been recorded, transmitted, and processed correctly. Data verification is the process of evaluating whether the data meets method, procedural, or contractual specifications. Data validation is the review of the quality of the data based on the specific DQIs indicated in this QAPP.

The sampler will perform data review for all field data initially before submitting to the laboratory. Upon submission to the laboratory, the laboratory will review the COC for completeness and document any non-conformances on the COC.

For the chemical laboratory data, the laboratory analyst will initially conduct the review, and the data will be peer reviewed by another analyst or capable reviewer. Data will be reviewed according to the laboratory QA Manual and the method specific SOP for data entry, calculations, and transformations as well review of quality control criteria. If deviations are noted, corrective actions will be taken with verification of both the reviewer and the original data collector. If consensus cannot be reached, the data will be rejected. During verification and validation of the data, all data that does not meet the DQIs listed in this QAPP will be qualified or rejected. A list of the type of qualifiers that may be applied to this data is listed in Table 14. All qualified data will be evaluated according to the actions listed.

TABLE 14 – DATA QUALIFIERS AND RESPONSE

Definition	Action To Be Taken
Analyte detected in associated Method Blank	Reject results. Indicates all, or a portion of, the amount found in a sample may be due to laboratory sources.
Diluted out	Accept results. Indicates a dilution to overcome matrix effects caused other analytes of interest to be diluted out of range. Normal quantitation is not available.
Holding time exceeded	Reject results. Method required holding time is exceeded.
Estimated value	Accept results when used to indicate result is below the project reporting limit, but above the Method Detection Limit (MDL).
Matrix Spike and/or Matrix Spike Duplicate Recovery outside acceptance limits	Acceptable results if associated Laboratory Control Sample is acceptable (No qualifier). Indicates matrix is adversely affecting the extraction or digestion of the analyte. If the Matrix Spike recovery is below acceptable limits, it may be likely that the reported results for the associated samples may be underestimated. Conversely, if the Matrix Spike results are high, it may be likely that the reported results for the associated samples may be overestimated.
Laboratory Control Sample outside acceptance limits	Reject results. Indicates that the laboratory system is out of control.
Sample received exceeding proper temperature or preservation criteria	Reject results. Indicates preservatives or temperature requirements have not been met and the bias on the sample result is unknown.
The analyte was analyzed for but not detected	Accept results. Indicates that the result is less than the reporting limit
Analyte exceeded calibration range	Accept results. Only reported in instances in which the calibration curve is exceeded and the sample cannot be reanalyzed.
Replicate / Duplicate precision outside of acceptance limits	Reject results, unless it occurs on a matrix spike duplicate. Indicates the precision is outside of normal acceptance criteria due to a lack of homogeneity or other factor.
Calibration criteria exceeded	Reject results. Indicates that the laboratory system is out of control.

D2. Validation and Verification Methods

The QA Manager will use the Field Sampling Audit Checklist and the Laboratory Data Checklist (Appendix A) to document the conformance of the data to this QAPP. This review will be submitted to the KDOW along with the data. The QA Manager will be responsible for making any final decisions concerning data quality and acceptability.

All final reports will receive an internal peer review to evaluate the content, calculations, and data analysis in the report. The reports will also undergo an internal grammatical review to look for grammatical errors and formatting. Lastly, the final report will receive a review from the Project Team prior to submission to the KDOW to ensure that all project objectives are achieved.

D3. Reconciliation with User Requirements and Data Quality Objectives
In the final report, descriptions of all relevant background information, summary, water body details, monitoring results, recommended solutions, and implementation plans will be detailed. Included in this document will be an overall assessment of the data quality and the uncertainty involved in the results.

SECTION E. - REFERENCES AND CITATIONS

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish.* Second Edition. EPA 841-B-99-002. USEPA, Office of Water, Washington, D.C.

Bunte, Kristin; Abt, Steven R. 2001. *Sampling surface and subsurface particle-size distributions in wadable gravel-and cobble-bed streams for analyses in sediment transport, hydraulics, and streambed monitoring*. Gen. Tech. Rep. RMRS-GTR-74. Fort Collins,CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 428 p.

Compton, M.C., G.J. Pond, and J.F. Brumley. 2003. *Development and application of the Kentucky Index of Biotic Integrity (KIBI)*. Kentucky Department for Environmental Protection, Division of Water, Frankfort, Kentucky.

Harrelson, C.C., C.L. Rawlins, and J.P. Potyondy. 1994. *Stream channel reference sites: An illustrated guide to field technique*. General Technical Report RM-245. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. 61p.

In-Situ Inc. 2006. *Level TROLL® Operator's Manual*. www.in-situ.com

Kentucky Division of Water (KDOW). 2004. *Project Final Report Guidelines for Clean Water Act §319(h)-Funded Projects*. Kentucky Energy and Environment Cabinet Department for Environmental Protection Division of Water Watershed Management Branch Nonpoint Source Section.

KDOW. 2008. *Methods for Assessing Biological Integrity of Surface Waters in Kentucky.* Kentucky Department of Environmental Protection.

KDOW. 2009a. *In-situ* Water Quality Measurements and Meter Calibration Standard Operating Procedure. Kentucky Department for Environmental Protection, Division of Water, Frankfort, Kentucky. DOWSOP03014

KDOW. 2009b. *Laboratory Procedures for Macroinvertebrate Processing and Taxonomic Identification and Reporting*. Kentucky Department of Environmental Protection.

KDOW. 2009c. *Methods for Sampling Benthic Macroinvertebrate Communities in Wadeable Waters*. Kentucky Department for Environmental Protection, Division of Water, Frankfort, Kentucky.

KDOW. 2010a. *2010 Integrated Report to Congress on the Condition of Water Resources in Kentucky: Volume II. 303(d) List of Surface Waters*. Kentucky Energy and Environment Cabinet Division. Frankfort, Kentucky.

KDOW. 2010b. *Measuring Stream Discharge Standard Operating Procedure*. Kentucky Department for Environmental Protection, Division of Water, Frankfort, Kentucky. DOWSOP03019

KDOW. 2011. *Sampling the Surface Water Quality in Lotic Systems*. Kentucky Department for Environmental Protection, Division of Water, Frankfort, Kentucky. DOWSOP03015

KDOW. Watershed Watch Water Chemistry Sampling Methods for Field Chemistry and Lab Analysis. http://www.lrww.org/training/chem-test.pdf

Kentucky Waterways Alliance and the Kentucky Division of Water. 2010. *Watershed Planning Guidebook for Kentucky Communities*. 1st ed. Kentucky Waterways Alliance and the Kentucky Division of Water.

LaMotte. Dissolved Oxygen Water Quality Test Kit Instruction Manual. Code 7414 / 5860. LaMotte Company. Chestertown, Maryland. www.lamotte.com

Lexington-Fayette Urban County Government (LFUCG). 2008. *Stormwater Quality Management Program (SWQMP) for Lexington-Fayette Urban County Government*. Tetra Tech, Inc, LFUCG Department of Public Works and Department of Environmental Quality.

Oakton Instruments. Waterproof TDSTestr and ECTestr Series Instructions. http://www.4oakton.com/Manuals/ConductivityTDS/TDS_ECTestrmnl.pdf

Price, David J. 2009. *Quality Assurance Plan (QAP) and Standard Operating Procedures (SOPs).* Lexington-Fayette Urban County Government Division of Water Quality Town Branch Laboratory.

Recker, S.A. and Meiman, J., 1990, Remedial investigations status report, Shop-N-Go Food Mart: Delta Environmental Consultants, Lexington, KY, unpublished report, 18 p.

Rosgen, D.L. 2008. *River Stability Field Guide*. Wildland Hydrology, Pagosa Springs, CO.

Spangler, L.E., 1992, Unpublished groundwater tracing data: University of Kentucky, Department of Geology.

United States and Commonwealth of Kentucky v. Lexington-Fayette Urban county Government, March 14, 2006, Consent Decree, Lodged in the United States District Court, Eastern District of Kentucky, Central Division at Lexington, Related to Civil Action No. 5:06-cv-00386. Accessed November 2010 at http://lexingtonky.gov/modules/ShowDocument.aspx?documentid=3572

United States Environmental Protection Agency (US EPA). March 2008, *Handbook for Developing Watershed Plans to Restore and Protect Our Waters*, Office of Water, Nonpoint Source Control Branch, EPA 841-B-08-002

APPENDIX A – DATASHEETS

Volunteer Monitoring Participant Agreement

Please fill this out, keep one and turn one in.

Please Print Name: **Best Phone**

Organization:

Shipping Address:

City State Zip **E-mail:**

I, to the best of my ability, will: (Check those that apply)

1. **___Conduct Field Chemistry. (D.O, pH, Conductivity, Temp)**
2. **___Collect Grab Samples,**
3. **___Take Photographs**
4. **___Read and follow the Project Quality Assurance Project Plan**
5. **___Return equipment when I "retire" from the project.**

6. **Equipment checked out to me: KIT#_____**

10. Lab Analysis Sample Sights Requested

ID#	Stream Name	Sampling Site (Map Reference)

11.___I understand and agree that the stream can be a dangerous place, the project organizers cannot protect me from slick banks, high water, snakes, falling timber and other hazards. I will ask permission before crossing private property. I will be on my guard and exercise due caution when handling chemicals. The information above is correct to the best of my knowledge.

SIGNED_____DATE_____

Lead Instructor Verification: This is to verify that the volunteer listed above has completed the modules I have initialed below:

Instructor Name(print)_____Date_____

Initial	Module:	Initial	Module:
	Grab Sample Collection		Project Logistics and Quality Assurance
	Field Chemistry		Habitat Assessment

Note to lead instructor: Return a copy of this form to your project registrar or to the Water Watch Office at 400 Fair Oaks Plaza Frankfort KY 40601 Att: JoAnn Palmer 1-800-928-0045 Ext 473

PHYSICAL CHARACTERIZATION/WATER QUALITY FIELD DATA SHEET (FRONT)

STREAM NAME	LOCATION	
STATION #_____ RIVERMILE_____	STREAM CLASS	
LAT _____ LONG _____	RIVER BASIN	
STORET#	AGENCY	
INVESTIGATORS		
FORM COMPLETED BY	DATE _____ TIME _____ AM PM	REASON FOR SURVEY

WEATHER CONDITIONS

Now
- ❑
- ❑
- ❑
- ❑_____%
- ❑

Past 24 hours
- ❑ storm (heavy rain)
- ❑ rain (steady rain)
- ❑ showers (intermittent)
- ❑_____%cloud cover
- ❑ clear/sunny

Has there been a heavy rain in the last 7 days? ❑ Yes ❑ No

Air Temperature_____° C

Other_____

SITE LOCATION/MAP

Draw a map of the site and indicate the areas sampled (or attach a photgraph)

STREAM CHARACTERIZATION

Stream Subsystem
❑ Perennial ❑ Intermittent ❑ Tidal

Stream Type
❑ Coldwater ❑ Warmwater

Stream Origin
- ❑ Glacial
- ❑ Non-glacial montane
- ❑ Swamp and bog
- ❑ Spring-fed
- ❑ Mixture of origins
- ❑ Other_____

Catchment Area_____km^2

PHYSICAL CHARACTERIZATION/WATER QUALITY FIELD DATA SHEET (BACK)

WATERSHED FEATURES	**Predominant Surrounding Landuse** ❑ Forest ❑ Commercial ❑ Field/Pasture ❑ Industrial ❑ Agricultural ❑ Other _____ ❑ Residential **Local Watershed NPS Pollution** ❑ No evidence ❑ Some potential sources ❑ Obvious sources **Local Watershed Erosion** ❑ None ❑ Moderate ❑ Heavy
RIPARIAN VEGETATION (18 meter buffer)	**Indicate the dominant type and record the dominant species present** ❑ Trees ❑ Shrubs ❑ Grasses ❑ Herbaceous **dominant species present** _____
INSTREAM FEATURES	**Estimated Stream Width** _____m **Estimated Stream Depth** _____m **Surface Velocity** _____m/sec **(at thalweg)** **Estimated Reach Length** _____m **Canopy Cover** ❑ Partly open ❑ Partly shaded ❑ Shaded **High Water Mark** _____m **Proportion of Reach Represented by Stream Morphology Types** ❑ Riffle_____% ❑ Run_____% ❑ Pool_____% **Channelized** ❑ Yes ❑ No **Dam Present** ❑ Yes ❑ No
AQUATIC VEGETATION	**Indicate the dominant type and record the dominant species present** ❑ Rooted emergent ❑ Rooted submergent ❑ Rooted floating ❑ Free Floating ❑ Floating Algae ❑ Attached Algae **dominant species present** _____ **Portion of the reach with aquatic vegetation** _____%
WATER QUALITY	**Temperature**_____° C **Specific Conductance**_____ **Dissolved Oxygen** _____ **pH** _____ **Turbidity** _____ **WQ Instrument Used** _____ **Water Odors** ❑ Normal/None ❑ Sewage ❑ Petroleum ❑ Chemical ❑ Fishy ❑ Other_____ **Water Surface Oils** ❑ Slick ❑ Sheen ❑ Globs ❑ Flecks ❑ None ❑ Other_____ **Turbidity (if not measured)** ❑ Clear ❑ Slightly turbid ❑ Turbid ❑ Opaque ❑ Stained ❑ Other_____
SEDIMENT/ SUBSTRATE	**Odors** ❑ Normal ❑ Sewage ❑ Petroleum ❑ Chemical ❑ Anaerobic ❑ None ❑ Other_____ **Oils** ❑ Absent ❑ Slight ❑ Moderate ❑ Profuse **Deposits** ❑ Sludge ❑ Sawdust ❑ Paper fiber ❑ Sand ❑ Relict shells ❑ Other_____ **Looking at stones which are not deeply embedded, are the undersides black in color?** ❑ Yes ❑ No

INORGANIC SUBSTRATE COMPONENTS (should add up to 100%)			**ORGANIC SUBSTRATE COMPONENTS (does not necessarily add up to 100%)**		
Substrate Type	**Diameter**	**% Composition in Sampling Reach**	**Substrate Type**	**Characteristic**	**% Composition in Sampling Area**
Bedrock			Detritus	sticks, wood, coarse plant materials (CPOM)	
Boulder	> 256 mm (10")				
Cobble	64-256 mm (2.5"-10")		Muck-Mud	black, very fine organic (FPOM)	
Gravel	2-64 mm (0.1"-2.5")				
Sand	0.06-2mm (gritty)		Marl	grey, shell fragments	
Silt	0.004-0.06 mm				
Clay	< 0.004 mm (slick)				

HABITAT ASSESSMENT FIELD DATA SHEET — HIGH GRADIENT STREAMS (FRONT)

STREAM NAME:	LOCATION:
STREAM WDTH (FT): DEPTH (FT):	PERENNIAL ☐ INTERMITTENT ☐ EPHEMERAL ☐
STATION #: RIVERMILE:	COUNTY: STATE:
LAT: LONG:	RIVER BASIN:
CLIENT:	PROJECT NO.
INVESTIGATORS/CREW:	

FORM COMPLETED BY:	DATE: TIME:	REASON FOR SURVEY:

Parameters to be evaluated in sampling reach

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
1. Epifaunal Substrate/ Available Cover	Greater than 70% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are <u>not</u> new fall and <u>not</u> transient.	40-70% mix of stable habitat; well suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	20-40% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 20% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2. Embeddedness	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3. Velocity/Depth Regime	All four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow). (Slow is < 0.3 m/s, deep is > 0.5 m.)	Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score low).	Dominated by 1 velocity/depth regime (usually slow-deep).
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
4. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills > 75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

HABITAT ASSESSMENT FIELD DATA SHEET — HIGH GRADIENT STREAMS (BACK)

Parameters to be evaluated in sampling reach

Habitat Parameter	Condition Category: Optimal	Suboptimal	Marginal	Poor
6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
7. Frequency of Riffles (or bends)	Occurrence of riffles relatively frequent; ratio of distance between riffles divided by width of the stream < 7:1 (generally 5 to 7); variety of habitat is key. In streams where riffles are continuous, placement of boulders or other large, natural obstruction is important.	Occurrence of riffles infrequent; distance between riffles divided by the width of the stream is between 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 to 25.	Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is a ration of > 25.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
8. Bank Stability (score each bank) Note: determine left or right side by facing downstream.	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. < 5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
SCORE: (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE: (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
9. Vegetative Protection (score each bank)	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or non-woody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
SCORE: (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE: (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters: little or no riparian vegetation due to human activities.
SCORE: (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE: (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

TOTAL SCORE:

Conductivity Survey Field Datasheet

Genral Info:

Stream Name _____ Segment ID _____

Date _____ Sampler Name _____ Cond Meter ID _____

Measurements to be made at least 100 ft apart in each segment starting upstream and working downstream.

Calibration:

Known Value 714 µS/cm Initial Calibration _____ µS/cm Final Calibration _____ µS/cm

Known Value 1438 µS/cm Initial Calibration _____ µS/cm Final Calibration _____ µS/cm

	Time (Indicate AM or PM)	Latitude N DD.DDDDDD	Longitude W DD.DDDDDD	Conductivity (µS/cm)	Temperature (°C)	Additional Comments
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						

Additional Notes:

Pebble Count Datasheet

Stream _____ Date _____

Station _____ Survey Crew _____

Size (in)	Particle	Description	Size (mm)	Particle Count				%	Cum %
				Riffle	Pool	Other	Total		
	SILT/CLAY	silt/clay	<0.062						
	SAND	very fine	0.062 - 0.125						
		fine	0.125 - 0.25						
		med	0.25 - 0.5						
		coarse	0.5 - 1						
0.04 - 0.08		very coarse	1 - 2						
0.08 - 0.16	**GRAVEL**	very fine	2 - 4						
0.16 - 0.22		fine	4 - 5.7						
0.22 - 0.31		fine	5.7 - 8						
0.31 - 0.44		med	8 - 11.3						
0.44 - 0.63		med	11.3 - 16						
0.63 - 0.89		coarse	16 - 22.6						
0.89 - 1.26		coarse	22.6 - 32						
1.26 - 1.77		very coarse	32 - 45						
1.77 - 2.5		very coarse	45 -64						
2.5 - 3.5	**COBBLE**	small	64 - 90						
3.5 - 5.0		small	90 - 128						
5.0 - 7.1		large	128 - 180						
7.1 - 10.1		large	180 - 256						
10.1 - 14.3	**BOULDER**	small	256 - 362						
14.3 - 20		small	362 - 512						
20 - 40		med	512 - 1024						
40 - 80		large	1024 - 2048						
	BEDROCK	bedrock							
	Total								

COC# | **CHAIN OF CUSTODY** | **Page 1 of 1**

Client: Third Rock Consultants, LLC
Project Name: Wolf Run Watershed Based Plan
Project # : KY10-030
Project Contact (sampling): Marcia L. Wooton
Phone # : 859-977-2000
Collected By:
Methodology Required: 40CFR Part 136

Turnaround Time Required: 30 Working Days

Comments:
Ammonia to RL of 0.05mg/L
Nitrite to RL of 0.03 mg/L

Conductivity Calibration:

Known: 714 µS/cm **Initial Calibration** _____µS/cm **Final** _____µS/cm
Known: 1438 µS/cm **Initial Calibration** _____µS/cm **Final** _____µS/cm

LFUCG
Division of Water Quality
Town Branch Laboratory
301 Lisle Industrial Avenue, Lexington, Kentucky, 40511
(859) 425-2416

Analytical Report to:
mwooton@thirdrockconsultants.com
Marcia L. Wooton
Third Rock Consultants, LLC
2526 Regency Road
Suite 180
Lexington, KY 40503
859-977-2000

* Matrix Code	* * Preservation Type			
SW - Surface Water	I, ST, SA	I, ST	I, ST	I
**** Preservative Code**	**Container Size/Type**			
SA - H2SO4 ST - Na2S2O3 I - Ice	8oz P	4oz P	4oz P	32oz P

Field Remarks:

Sample I.D.	Sample Location	Matrix *	Collection Date	Collection Time	Grab / Comp	Filt'd Y/N	# of Containers Per Analysis: Ammonia	*E. coli*	Fecal coliform	Alkalinity, TSS, TDS, Hardness, Nitrite	On-Site/Field Measurements: Turbidity (Visual) *1=clear, 2=slightly turbid, 3=turbid, or indicate if "other"*	Dissolved Oxygen (mg/L)	pH (S.U.)	Specific Conductance (umho/cm)	Temperature (°C)
W01-11 __ __ __ __	Wolf Run @ Old Frankfort Pike	SW			G	N	1	1	1	1					
W02-11 __ __ __ __	McConnel Branch @ Prestons Cave	SW			G	N	1	1	1	1					
W03-11 __ __ __ __	Wolf Run @ Valley Park	SW			G	N	1	1	1	1					
W04-11 __ __ __ __	Vaughn's Branch @ Valley Park	SW			G	N	1	1	1	1					
W05-11 __ __ __ __	Cardinal Run @ Devonport Dr	SW			G	N	1	1	1	1					
W06-11 __ __ __ __	Wolf Run @ Wolf Run Park	SW			G	N	1	1	1	1					
W07-11 __ __ __ __	Vaughn's Branch @ Pine Meadow Park	SW			G	N	1	1	1	1					
W08-11 __ __ __ __	Vaughn's Branch @ Picadome	SW			G	N	1	1	1	1					
W09-11 __ __ __ __	Wolf Run @ Faircrest Dr	SW			G	N	1	1	1	1					
W10-11 __ __ __ __	Springs Branch @ Faircrest Drive	SW			G	N	1	1	1	1					
W11-11 __ __ __ __	Big Elm Tributary @ Harrodsburg Rd	SW			G	N	1	1	1	1					
W12-11 __ __ __ __	Wolf Run @ Lafayette Pkwy	SW			G	N	1	1	1	1					
WDD-11 __ __ __ __	Duplicate	SW	----	----	G	N	1	1	1	1	See Field Notes for Duplicate ID				

Relinquished By:	Date / Time	Received By:	Date / Time

Temp. Upon Receipt (C): _____ **Measured By:**_____
Containers Properly Preserved: (Yes / No)
Headspace: (Yes / No / NA)
Bottles Intact: (Yes / No)
COC Seals Intact: (Yes / No / NA)
Additional Documentation Attached: (Yes / No)

Original COC To Laboratory (Accompany Samples & Report) **COC Copy - TRC Project File** **COC Copy - TRC Laboratory Services Coordinator**

sje 04/11/11

COC#	CHAIN OF CUSTODY	Page 1 of 1

Client: Third Rock Consultants, LLC
Project Name: Wolf Run Watershed Based Plan
Project # : KY10-030
Project Contact (sampling): Marcia L. Wooton
Phone # : 859-977-2000
Collected By:
Methodology Required: 40CFR Part 136

KENTUCKY GEOLOGICAL SURVEY
Laboratory Services
COAL • WATER • MINERALS
228 Mining & Mineral Resources Building, University of Kentucky
Lexington, Kentucky, 40506-0107
(859) 323-0555

Analytical Report to:
mwooton@thirdrockconsultants.com
Marcia L. Wooton
Third Rock Consultants, LLC
2526 Regency Road
Suite 180
Lexington, KY 40503
859-977-2000

Turnaround Time Required: 30 Working Days

Comments:
Ortho-Phosphorus to RL of 0.05 mg/L
Phosphorus to RL of 0.02 mg/L
Nitrate to RL of 0.03 mg/L
TKN to RL of 0.5 mg/L

* Matrix Code	* * Preservation Type		
SW - Surface Water	I, FF, SA	I	I, SA
** Preservative Code	Container Size/Type		
FF - Field Filter HA - HCl NA - HNO3 SA - H2SO4 SH - NaOH ST - Na2S2O3 I - Ice	8oz P	8oz P	32oz P
	Requested Lab Analysis		
	Ortho-phosphorus	Nitrate	Total phosphorus, TKN

Field Remarks:

Sample I.D.	Sample Location	Matrix *	Collection Date	Collection Time	Grab / Comp	Filt'd Y/N	# of Containers Per Analysis			Comments
W01-11 __ __ __ __	Wolf Run @ Old Frankfort Pike	SW			G	N	1	1	1	
W02-11 __ __ __ __	McConnel Branch @ Prestons Cave	SW			G	N	1	1	1	
W03-11 __ __ __ __	Wolf Run @ Valley Park	SW			G	N	1	1	1	
W04-11 __ __ __ __	Vaughn's Branch @ Valley Park	SW			G	N	1	1	1	
W05-11 __ __ __ __	Cardinal Run @ Devonport Dr	SW			G	N	1	1	1	
W06-11 __ __ __ __	Wolf Run @ Wolf Run Park	SW			G	N	1	1	1	
W07-11 __ __ __ __	Vaughn's Branch @ Pine Meadow Park	SW			G	N	1	1	1	
W08-11 __ __ __ __	Vaughn's Branch @ Picadome	SW			G	N	1	1	1	
W09-11 __ __ __ __	Wolf Run @ Faircrest Dr	SW			G	N	1	1	1	
W10-11 __ __ __ __	Springs Branch @ Faircrest Drive	SW			G	N	1	1	1	
W11-11 __ __ __ __	Big Elm Tributary @ Harrodsburg Rd	SW			G	N	1	1	1	
W12-11 __ __ __ __	Wolf Run @ Lafayette Pkwy	SW			G	N	1	1	1	
WDD-11 __ __ __ __	Duplicate	SW	----	----	G	N	1	1	1	See Field Notes for Duplicate ID

Relinquished By:	Date / Time	Received By:	Date / Time

Temp. Upon Receipt (C): ___ Measured By:_____
Containers Properly Preserved: (Yes / No)
Headspace: (Yes / No / NA)
Bottles Intact: (Yes / No)
COC Seals Intact: (Yes / No / NA)
Additional Documentation Attached: (Yes / No)

Original COC To Laboratory (Accompany Samples & Report) **COC Copy - TRC Project File** **COC Copy - TRC Laboratory Services Coordinator**

sje 04/11/11

THIRD ROCK CONSULTANTS

Macroinvertebrate Sample Chain of Custody
Project Information Sheet

Client Name:_____ Project Administrator:_____ Project Number:_____ Due Date:_____

Sampling Site Location/Stream Name:_____ County:_____ State:_____

System Type:_____ EcoRegion:_____ Total Number of Samples:_____ Total Number of Containers:_____

Reporting Requirements: __ Laboratory Data Sheet; __ Excel Spreadsheet; __ MBI Calculations via : __ e-Submittal; __ Hardcopy; __ Both

Samples Relinquished By: _____ Date/Time: _____ Sample Received By: _____ Date/Time: _____

Samples Relinquished By: _____ Date/Time: _____ Sample Received By: _____ Date/Time: _____

Comments/Special Instructions: _____

Sample Reference ID	Qualitative or Quantitative	Latitude	Longitude	Collected By	Collection Date	Sample Type	Field Preservative	# of Containers Per Sample

- Continue on Reverse for More Samples -

System Type: Headwater Stream; Wadeable Stream; Large River; Lotic; Other _____
EcoRegion: Bluegrass; Mountain; Pennyroyal; Mississippi Valley-Interior River Lowlands; Other _____
Sample Type: **KN** KickNet; **TK** Traveling Kick; **MH** Multihabitat; **S** Surber; **HD** Hester-Dendy Multiplate; **HDD** HD Deep; **HDS** HD Shallow; **OT** Other_____; **NA** Not Available

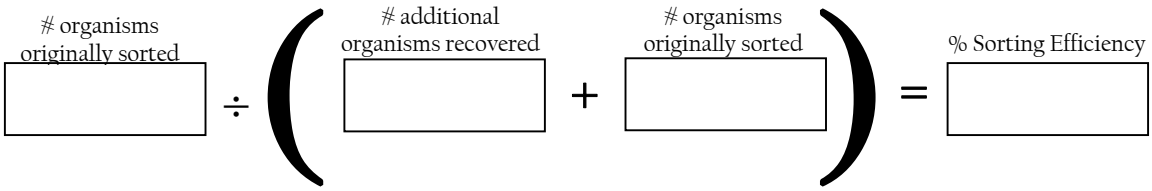
MacLIMS: Client Setup/Login By _____ Date _____; Reported By _____ Date _____; Invoiced By _____ Date _____

Sample Reference ID	Qualitative or Quantitative	Latitude	Longitude	Collected By	Collection Date	Sample Type	Field Preservative	# of Containers Per Sample

Third Rock Consultants, LLC
Macroinvertebrate Sample Sorting Efficiency Form

Client Name: _____
SampleID: _ _____
Third Rock Project #: _____

Original Sorter:	Resorted By:
Date Sorted:	Date Resorted:
# of Grids Sorted:	# of Grids Sorted:
# of Organisms Originally Sorted:	# Additional Organisms Recovered:



Additional Organisms Located

Taxon	Number
Total:	

Comments:

Reviewed By: _____ *Date:* _____

Third Rock Consultants, LLC
Macroinvertebrate Sample Taxonomic & Enumeration Efficiency Form

Client Name: _____
Sample ID: _____
Third Rock Project #: _____

Original Taxonomist:	Second Taxonomist:
Original Date Completed:	Review Date Completed:
# Organisms Enumerated (*Taxonomist 1*):	# Organisms Enumerated (*Taxonomist 2*):

Percent Difference in Enumeration (PDE) =

$$(n_1 - n_2) \div (n_1 + n_2) \times 100 = \% \text{ Difference in Enumeration (PDE)}$$

n_1 = *# organisms counted by Taxonomist 1*
n_2 = *# organisms counted by Taxonomist 2*

Percent Taxonomic Disagreement (PTD) =

$$PTD = [1 - (comp_{pos} \div N)] \times 100$$

$Comp_{pos}$ = *number of taxonomic agreements (see Taxonomic Comparison Form)*
N = *total number of organisms*

Comments:

Reviewed By: _____ *Date:* _____

Third Rock Consultants, LLC
Macroinvertebrate Sample Taxonomy Precision Form

Client Name: _____
Sample ID: _____
Third Rock Project #: _____

Taxon	Taxonomist 1	Taxonomist 2	# Agreements

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1	8	0	9	4	2	5	2	5	8	2	4	7	1	3	4	7	7	4	3	3	3	6	2	0	1	8	9	7	2	1	3	4
2	3	5	6	3	2	1	9	8	8	2	1	1	9	0	4	5	2	6	1	8	2	7	5	1	2	6	2	7	1	0	9	5
3	1	3	3	0	6	3	3	1	3	7	5	3	9	6	9	3	8	7	3	8	6	8	1	5	1	5	3	8	8	5	4	3
4	3	5	6	5	0	0	1	6	2	2	4	3	6	4	3	2	4	7	9	6	6	0	9	5	5	2	8	3	1	6	2	0
5	7	8	5	0	5	9	2	5	5	5	8	8	7	3	1	1	2	1	9	2	4	5	4	5	3	5	3	0	5	5	8	9
6	4	4	9	0	5	4	1	7	9	7	2	7	6	1	5	3	5	9	0	1	4	8	7	8	9	9	8	0	9	8	7	7
7	6	5	4	5	9	1	0	4	9	3	1	8	8	8	1	9	7	5	3	7	2	7	8	5	9	3	7	3	2	4	4	5
8	3	6	2	8	5	9	9	5	1	2	1	5	9	7	5	3	9	2	2	3	5	6	5	8	2	9	4	4	2	8	9	9
9	4	6	6	5	4	8	2	0	7	5	5	4	0	6	1	2	9	6	8	3	4	2	5	1	9	1	3	8	1	7	0	9
10	6	4	9	8	7	5	1	9	0	4	7	4	7	8	1	8	6	8	3	2	9	6	8	3	9	8	7	2	4	0	9	0
11	6	7	2	2	9	8	6	9	9	3	6	1	7	8	7	5	4	8	8	3	1	3	1	5	9	6	7	9	8	8	3	4
12	9	7	4	8	5	9	3	2	5	1	1	5	2	7	2	1	0	0	3	3	9	3	0	3	9	7	1	3	4	0	1	2
13	5	6	4	1	1	4	1	7	1	4	1	9	7	4	3	4	8	1	6	5	7	3	6	8	1	2	1	8	5	0	3	9
14	7	4	4	4	9	2	0	0	8	8	4	0	5	8	8	2	4	3	9	8	3	9	0	4	9	1	9	9	9	3	3	6
15	8	2	7	9	3	0	1	9	4	6	7	2	3	7	4	3	3	9	7	9	4	6	8	9	9	0	2	1	6	9	9	0
16	0	1	6	1	7	6	1	7	1	0	2	4	2	3	8	7	2	8	9	1	6	6	7	7	1	5	8	5	2	4	8	2
17	7	3	8	8	9	7	5	9	7	5	5	5	6	6	2	4	9	9	7	7	2	0	0	8	5	5	9	6	9	7	4	0
18	7	8	3	0	4	7	1	4	3	6	9	5	2	9	1	9	1	8	0	4	4	0	4	4	1	0	3	4	2	5	9	7
19	9	8	8	7	4	2	1	6	6	5	2	6	4	5	3	5	8	4	3	0	5	2	7	0	9	6	0	5	0	7	6	8
20	1	2	6	1	2	5	1	6	8	5	6	9	2	3	1	0	3	9	3	9	8	7	0	3	9	8	4	1	0	3	5	3
21	3	9	4	7	4	9	3	7	7	6	3	4	2	5	4	3	6	2	3	9	7	4	5	5	2	0	5	5	7	7	9	5
22	4	5	5	0	8	1	0	3	1	2	5	0	2	3	0	4	1	1	3	8	9	7	8	8	9	1	4	4	4	5	2	6
23	1	3	4	4	9	6	9	7	2	3	8	3	6	9	7	6	6	2	5	1	4	2	0	1	2	0	3	8	6	5	5	2
24	8	9	7	6	5	8	2	3	8	4	8	7	0	4	5	0	3	1	0	6	9	1	6	6	2	7	1	7	7	6	0	1
25	7	7	1	0	9	9	4	3	6	9	7	8	8	2	7	3	9	7	1	4	9	7	0	0	1	5	6	6	2	8	8	9
26	6	9	5	9	6	0	0	8	8	4	4	2	2	2	8	2	1	5	2	4	2	5	1	7	5	8	1	8	0	0	8	1
27	7	9	4	1	2	3	1	2	2	4	3	1	6	7	0	2	9	9	8	4	3	4	6	9	3	0	8	5	4	7	6	2
28	2	2	8	4	0	8	9	6	9	1	0	7	5	5	4	2	7	3	1	9	3	7	8	2	1	0	6	8	9	5	7	4
29	9	5	9	4	7	4	1	6	9	3	6	5	6	0	4	5	1	1	8	3	5	9	1	6	9	5	9	9	1	1	4	3
30	4	6	1	3	8	5	4	9	6	3	6	9	3	2	0	8	5	1	0	9	9	6	8	0	1	1	6	8	6	1	3	3

Field Sampling Audit Checklist

General Information:

Project No. / Description:_____ Date:_____ Field Personnel:_____ Reviewer: _____
Methods Reviewed: Associated SOP, manual, or quality standard:

Evaluation:
For each DQI, indicate whether the field personnel met the project objectives. Document in the comments columns the actual specification for that DQI and explain any non-conformances.

Data Quality Indicator (DQI)	Conforms?*		Comments
	Y	N	
Precision Agreement among repeated measurements of the same property under identical, or substantially similar conditions, random error. (i.e. duplicates)			
Bias Systematic error or persistent distortion of a measurement in one direction			
Accuracy Overall agreement of a measurement to a known value; includes a combination of precision and bias. (i.e. difference from known)			
Representativeness Qualitative term expressing the degree to which a portion accurately and precisely represents the whole (i.e. where sampled, conditions)			
Completeness Amount of valid data needed to be obtained (Ensure all data is recorded and all datasheets are filled out in their entirety)			
Sensitivity Capability to discriminate between measurement responses representing different levels of variable interest (i.e. reporting limit, lowest unit of measure)			
Interference Elimination of distorting or inhibiting effects on the measurement			
Training Documented evidence of meeting all necessary training requirements			

*Yes / No – Indicates that the task performed in conformance / out of conformance with the project's DQI. If the DQI is not relevant to the task, write N/A in the comments column.

Field Sampling Audit Checklist

Water Quality Sample Collection:

Data Quality Indicator (DQI)	Conforms?*		Comments
	Y	N	
Samples correctly labeled?			
Storage and preservation?			

Equipment Used:

Equipment Name	Last Calibration By / Date	Conforms?	Comments

Other Comments:

*Yes / No – Indicates that the task performed in conformance / out of conformance with the project's DQI. If the DQI is not relevant to the task, write N/A in the comments column.

Laboratory Data Quality Checklist

General Information:

Project No. / Description:_____	Date:_____
Laboratory: _____	Reviewer: _____
Data Reviewed:	

Evaluation:

For each DQI, indicate whether the laboratory data met the project objectives. If not, document non-conformances in the comments column and the action to be taken by data users. Attach additional sheets if necessary for documentation.

Data Quality Indicator (DQI)	Conforms?*		Comments
	Y	N	
Precision Agreement among repeated measurements of the same property under identical, or substantially similar conditions, random error. (i.e. duplicates, splits)			
Accuracy Overall agreement of a measurement to a known value; includes a combination of precision and bias. (i.e. difference from known laboratory control standard of matrix spike)			
Sensitivity Capability to discriminate between measurement responses representing different levels of variable interest (i.e. reporting limit, method blank contamination)			
Completeness Amount of valid data required (Ensure all data is reported with a useable result)			
Preservation/Handing Analyzed within hold time and properly preserved			
Interference Elimination of distorting or inhibiting effects on the measurement			
Other Describe non-conformance			

*Yes / No – Indicates that the is in conformance / out of conformance with the project's DQI. If the DQI is not relevant to the data, write N/A in the comments column.

APPENDIX B – STANDARD OPERATING PROCEDURES

Lexington-Fayette Urban County Government

Division of Water Quality

Town Branch Laboratory

Quality Assurance Plan (QAP)

and

Standard Operating Procedures (SOPs)

July 8, 2008
Revised September 16, 2009

David J. Price, Ph.D.
Laboratory Supervisor

Table of Contents

FORWARD 1
Section 1 2
Quality Policy Statement 2
1.1 Quality Policy Statement 2
1.2 Specific Objectives 2
1.3 QAP Availability 2
Section 2 3
Laboratory Organization and Staff Responsibilities 3
2.1 Laboratory Organization 3
2.2 Staff Responsibilities 3
2.3 Current Personnel 3
Section 3 4
Organizational Charts 4
3.1 Laboratory's Place in Company and Laboratory Organization 4
3.2 Laboratory's Organization 4
Section 4 5
4.1 Scope 5
4.2 QAP Documentation Control Procedures 5
4.3 SOP Documentation Control Procedures 5
4.4 Forms/Data Sheets Documentation Control Procedures 5
Section 5 6
Laboratory's Approved Signatories 6
5.1 Approved Signatories 6
Section 6 7
General Quality Control Procedures and Practices 7
6.1 Documentation of Test Methods and Laboratory Practices 7
6.2 General Quality Control Guidelines 7
6.3 Method Blanks 7
6.4 Matrix Spikes 8
6.5 Laboratory Control Samples 8
6.6 Matrix Spike Duplicates and Sample Duplicates 9
6.7 Quality Control Charts and Tabulations 9
6.8 Initial Demonstration of Method Performance Studies (IDMP) 10
6.9 Method Detection Limits (MDL) 11
6.10 Internal Audits 11
6.11 Analytical Quality Control Standards 12
6.12 Reagents 12
6.13 Glassware 12
6.14 Laboratory Pure Water 12
Section 7 13
Verification Practices 13
7.1 Performance Evaluation (PE) Testing 13
7.2 Standard Reference Materials 13
7.3 Internal Quality Control Programs 13

Section 8 15
Equipment Procedures for Calibration, Verifications, and Maintenance 15
8.1 Equipment Calibration 15
8.2 Balances 16
8.3 pH Meters 16
8.4 Conductivity Meters 16
8.5 Thermometers 16
8.6 Refrigerators 16
8.7 Incubators 17
8.8 Ovens 17
8.9 Other Laboratory Equipment 18
Section 9 19
Test Methods and SOPs 19
9.1 Scope 19
9.2 Standard Operating Procedures (SOPs) 19
Section 10 20
The Laboratory's Physical Facilities Including Services and Resources 20
Section 11 22
Procedures for Reviewing New Work 22
11.1 Scope 22
11.2 Unknown Samples 22
11.3 Contract Analyses 22
Section 12 23
Sample Acceptance and Receipt Policy 23
12.1 Scope 23
12.2 Sample Acceptance Requirements 23
12.3 Verification of Preservation 25
12.4 Actions for Deviations from the Lab's Sample Acceptance Requirements 25
12.5 Laboratory Log of Received Samples 26
Section 13 27
Sample Tracking and Storage Procedures 27
13.1 Scope 27
13.2 Sample Tracking Record 27
13.3 Proper Storage and Avoidance of Cross-contamination of Samples 27
13.4 Security of Samples 27
13.5 Sample Disposal 27
Section 14 28
Record Keeping, Data Review and Reporting Procedures 28
14.1 Scope 28
14.2 Records Maintained by the Laboratory 28
14.3 Records of Standards and Analytical Reagents 29
14.4 Computerized Data Storage 30
14.5 Administrative Records 30
14.6 Laboratory Record Entries and Change of Entries 30
14.7 Record Retention 31
14.8 Raw Data Associated with Sample Analysis 31

14.9 Test Data Reports 31
14.10 Electronic Data Transfer 32
14.11 Sample Disposal Records 32
14.12 Waste Disposal 32
Section 15 33
Corrective Action Policies and Procedures 33
15.1 Scope 33
15.2 Identification of Discrepancies 33
15.3 Staff Responsible for Investigation and Corrective Action 33
15.4 Documentation and Review of Corrective Action 34
Section 16 35
Procedures for Permitting Departures from Documented Policies and Procedures 35
16.1 Scope 35
16.2 Requests for and Approval of Departures 35
16.3 Use of Analytical Methods Not Approved by KYDEP or U.S. EPA 35
16.4 Reporting of Non-compliant Data 35
Section 17 36
Procedures for Dealing with Complaints 36
17.1 Scope 36
17.2 Complaint Receipt Procedures 36
17.3 Complaint Investigation Procedures 36
17.4 Complaint Response Procedures 36
Section 18 37
Procedures for Protecting Confidentiality and Proprietary Rights 37
18.1 Procedure for Protecting Confidentiality 37
18.2 Examples of Confidential Data 37
18.3 Procedure for Open-Records Requests 37
Section 19 38
Procedures for Internal Audits 38
19.1 Annual Internal Audit 38
19.2 Corrective Actions Regarding the Annual Internal Audit 38
19.3 Notification of Invalid Data 38
Section 20 39
20.1 Scope 39
20.2 Correcting Deficiencies in the QAP 39
Section 21 40
Training and Personnel Requirements 40
21.1 Scope and Training Objectives 40
21.2 Training Required for Laboratory Supervisor 40
21.3 Training Required for Quality Assurance Officer 40
21.4 Training Required for Technicians 40
21.5 Additional Training for Technicians 41
21.6 Substituting Experience or Education to Meet Training Requirements 41
21.7 Instrument Training Requirements 41
21.7 Training Records 41
Section 22 42

Glossary 42
Section 23 51
Bibliography 51
APPENDIX A LABORATORY ANALYSIS LIST A-1
Table A1. Town Branch Laboratory Analysis List A-2
Table A2. West Hickman WWTP Analysis List A-5
Table A3. Blue Sky WWTP Analysis List A-7
Table A4. Data Provided to the Laboratory by Operations Staff A-8
APPENDIX B STAFF RESPONSIBILITIES B-1
APPENDIX C CURRENT PERSONNEL AND LAB APPROVED SIGNATURES C-1
TOWN BRANCH LABORATORY STANDARD OPERATING PROCEDURES 1
Alkalinity (Titrimetric) 2
Biochemical Oxygen Demand (BOD_5) 15
Total Residual Chlorine Analysis 22
Free Cyanide CN (F) 27
Total Cyanide CN (T) 30
Chromium, Total and Hexavalent (Cr^{6+}) 34
Dissolved Oxygen Analysis (D.O.) 39
Fecal Coliform - Membrane Filter Procedure 44
m-ColiBlue24 Method for the Determination of Total Coliforms and *E. coli* 54
Hardness, Total (mg/L as $CaCO_3$; Titrimetric, EDTA) 60
pH (Electrometric) 63
Total Phosphorus Analysis 69
Ascorbic Acid Method 69
Settleable Solids 78
Total Suspended Solids (TSS) 81
Total Solids 85
Volatile Acids 89
Volatile Solids 94
WEST HICKMAN CREEK SOPs 1
WH Dissolved Oxygen Field Analysis (D.O.) 2
WH pH (Electrometric) 7
WH Total Residual Chlorine Analysis 12
WH Fecal Coliform - Membrane Filter Procedure 17
WH Total Phosphorus Analysis 24
APPENDIX D LABORATORY BENCHSHEETS D-1

FORWARD

The following Quality Assurance Plan (QAP) for the Town Branch Laboratory has been adapted from the Illinois Water Environment Association Laboratory Committee Model QAP. This document is intended as guidance only and not a detailed explanation of the accreditation rules. Periodic review of this document will be conducted and updates will be made as needed to keep this document up to date and relevant. The Town Branch Laboratory, as part of the Lexington-Fayette Urban County Government (LFUCG), prepared this document to serve as a clearinghouse of information related to the operation of the Laboratory housed at Town Branch WWTP. The Laboratory serves the:

- Town Branch WWTP KPDES Permit No. KY0021491
- West Hickman WWTP KPDES Permit No. KY0021504
- Blue Sky WWTP KPDES Permit No. KY0027286

The laboratory is operated in accordance with the Federal Register 40 CFR Part 122, 136, et al. Primary references include:

- EPA – 600/4-79-020. Methods for Chemical Analysis of Water and Wastes. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

- EPA – 600/8-78-017. Microbiological Methods for Monitoring the Environment: Water and Wastes. U.S. Environmental Protection Agency; Environmental Monitoring and Support Laboratory, Office of Research and Development, Washington, DC, 1978.

- Standard Methods for the Examination of Water and Wastewater. APHA-American Public Health Association. Standard Methods. 21st edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

Section 1
Quality Policy Statement

1.1 Quality Policy Statement

This Quality Assurance Plan (QAP) provides a written plan of operation for the laboratory that allows for accuracy, precision, and reliability of laboratory analyses and that data produced in the laboratory meets or exceeds user requirements. Good Laboratory Practices are employed to maximize data reliability.

1.2 Specific Objectives

The laboratory will employ methods capable of meeting user's needs for accuracy, precision, sensitivity, and specificity. Whenever possible, analytical methods used will be those approved by regulatory or accrediting authorities.

Under the supervision of the Laboratory Management, laboratory staff members will receive training in quality technology of sufficient depth to perform their assigned duties.

The laboratory will establish a level of quality for routine performance to use as a baseline from which to measure quality improvement efforts.

Laboratory staff will monitor routine operational performance through analysis of appropriate quality control solutions and through participation in inter-laboratory testing programs. The laboratory will provide for corrective actions as necessary

The laboratory will operate in conformance with requirements established by the State of Kentucky and/or the USEPA.

1.3 QAP Availability

Copies of the laboratory's QAP are available from:

Dr. David J. Price, Ph.D., Laboratory Supervisor

Mrs. La Vada M. Green, QA/QC Manager

Section 2
Laboratory Organization and Staff Responsibilities

2.1 Laboratory Organization

The Laboratory operates as a department of the Division of Water Quality for the Lexington-Fayette Urban County Government (LFUCG). The laboratory performs analyses on samples from the Town Branch WWTP, the West Hickman WWTP, the Blue Sky WWTP, several industrial pretreatment samples, and samples brought in from other outside sources. A list of samples and analyses performed are presented in Appendix A.

2.2 Staff Responsibilities

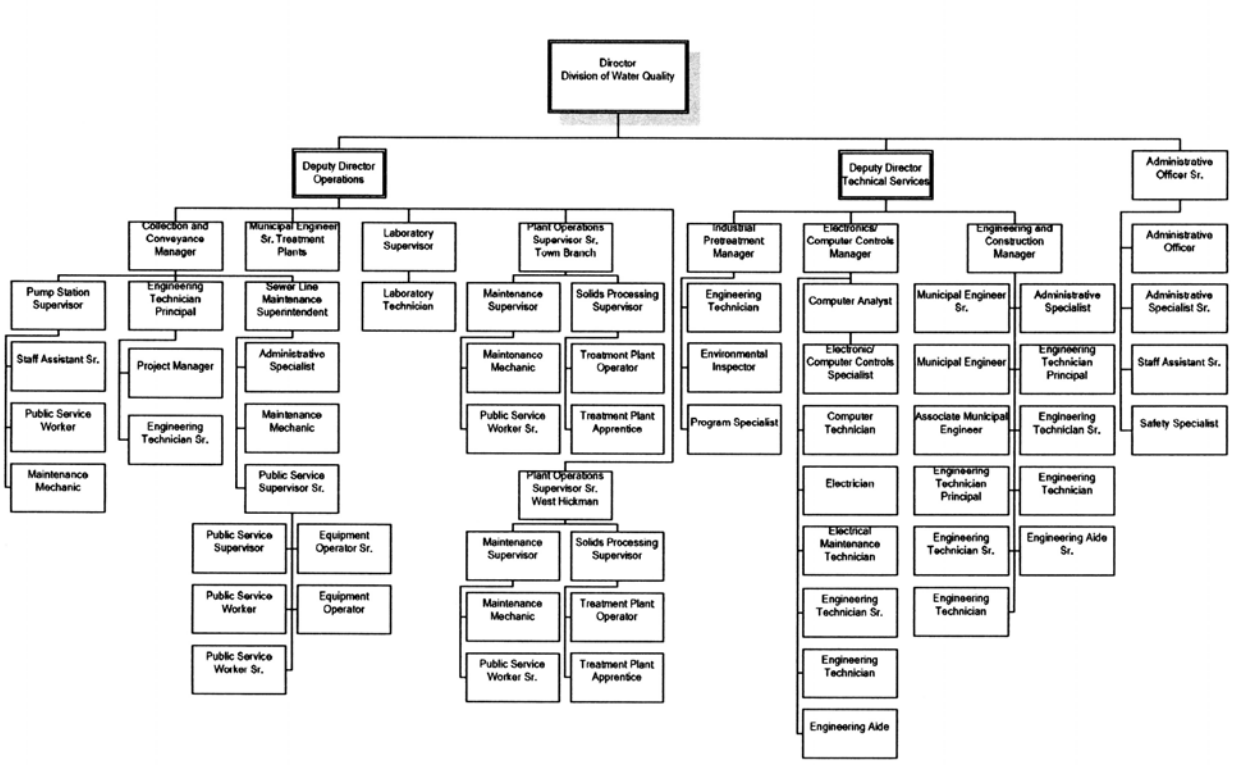
Appendix B contains job descriptions and responsibilities for all laboratory positions.

2.3 Current Personnel

Appendix C lists the laboratory's positions, names of personnel, education, and approved signatures.

Section 3
Organizational Charts

3.1 Laboratory's Place in Company and Laboratory Organization



3.2 Laboratory's Organization

3.2.1 Mrs. La Vada Green - QA/QC Manager
3.2.2 Mr. DiLinh Cao – Laboratory Safety
3.2.3 Mrs. Maria Lundin – Microbiology
3.2.4 Mr. Brian Reynolds – Database administration

Section 4
Documentation Control and Maintenance Procedures

4.1 Scope

The laboratory maintains document control procedures for its QAP and all standard operation procedures (SOPs) including analytical procedures and sample preparation procedures.

4.2 QAP Documentation Control Procedures

4.2.1 Each page of the QAP includes the title, revision number, effective date, and page number.

4.2.2 Copies of previous versions of the QAP are archived and kept by laboratory management and are subject to the record keeping requirements in Section 14.

4.2.3 When minor revisions are made to a section of the QAP, the updated section is added to the QAP and the previous version is removed and archived for a minimum of five years.

4.2.4 Archived information shall be available to regulatory agencies.

4.3 SOP Documentation Control Procedures

4.3.1 All SOPs are assigned a unique name or code.

4.3.2 Each SOP document contains a revision number.

4.3.3 Each page of a SOP includes its unique name or code, revision number, effective date, and current page number of total pages in section.

4.3.4 Copies of previous versions of SOPs are archived and kept by laboratory management for a minimum of five years. These are subject to the record keeping requirements in Section 14.

4.3.5 SOPs are available to laboratory personnel as part of a comprehensive lab manual. New SOPs are added to the lab manual as they are issued. When SOPs are revised, the revision is added to the lab manual and the previous version is removed and archived. Likewise, any procedure no longer in effect is removed from the lab manual and archived for a minimum of five years.

4.4 Forms/Data Sheets Documentation Control Procedures

All forms and data sheets prepared by the laboratory display a title, version number, and effective date.

Section 5
Laboratory's Approved Signatories

5.1 Approved Signatories

The laboratory's approved signatures with job titles and accreditation position are shown in Appendix 3. Also, the QAP title page has signed concurrence with appropriate titles of all responsible parties, including the quality assurance officer and laboratory director.

_____
Laboratory Supervisor

_____
Quality Assurance Officer

_____
Town Branch Superintendent

_____
West Hickman Creek Superintendent

_____
Director, Division of Water Quality

Section 6
General Quality Control Procedures and Practices

6.1 Documentation of Test Methods and Laboratory Practices

6.1.1 All test methods have written standard operating procedures (SOPs). The written methods describe each procedure and the equipment needed. Each SOP follows a standard format, which includes additional information on quality control measures and acceptance criteria for data.

6.1.2 All laboratory practices pertaining to QA/QC as well as laboratory operation practices other than analytical methods have written standard operating procedures.

6.2 General Quality Control Guidelines

6.2.1 For each test method, the quality control measures described in this QAP are followed wherever applicable. Additional test method quality control measures may be implemented, providing they are more stringent than those in this QAP.

6.2.2 All quality control protocol and test procedures are assessed and evaluated on an on-going basis.

6.2.3 Quality control procedures follow the direction provided in the test method SOP for evaluation of results; and accept, reject, or qualify sample data based upon the acceptance criteria specified in the test method. The laboratory establishes the evaluation procedure and acceptance criteria for a quality control procedure when not specified by the test method. Whether specified in the test procedure or established by the laboratory, the evaluation procedures and the acceptance criteria are documented either in this QAP or in the test method SOP.

6.2.4 If a quality control procedure results in the laboratory rejecting or qualifying sample data, the problem is investigated, appropriate corrective action implemented, and the incident documented.

6.3 Method Blanks

6.3.1 Method blanks are prepared and analyzed with each batch of environmental samples and are carried through the entire analytical process.

6.3.2 The method blank is acceptable if it does not contain an analyte of interest at a concentration greater than the highest of the following: (a) The MDL of the regulatory limit for that analyte; (b) the MDL of the measured concentration for that analyte in any environmental sample in the batch; or (c) categorical limits, such as found in BOD analysis.

6.3.3 When method blank acceptance criteria are not met, any non-detect results in the associated batch of environmental samples are reported with qualification.

6.3.4 Section 15, Corrective Action Policies and Procedures, references procedures for taking corrective actions when blanks do not meet acceptance criteria.

6.4 Matrix Spikes

6.4.1 For all test methods performed by the laboratory in which materials suitable for matrix spiking are available, matrix spikes are performed at a rate of one per sample set of similar matrix type, per sample extraction or preparation procedure.

6.4.2 The laboratory utilizes the spiking analytes specified in the test method. When the test method indicates that all method analytes are to be matrix spiked, then the laboratory spikes all analytes of interest.

6.4.3 Samples are selected on a rotating basis for matrix spike analysis from among various waste streams, monitoring locations and other applicable locations.

6.4.4 Procedures used to select samples and analytes for spiking are documented.

6.4.5 Each analytical SOP references quality control criteria to use in determining discrepancies and accepting data when matrix spikes do not meet acceptance criteria.

6.4.6 Section 15, Corrective Action Policies and Procedures, references procedures for taking corrective actions when matrix spikes do not meet acceptance criteria.

6.5 Laboratory Control Samples

6.5.1 For each test method, laboratory control samples (LCS) are analyzed at a minimum of one per batch.

6.5.2 The results of the LCS analyses are used to determine batch acceptance.

6.5.3 Standards for preparing LCS samples are obtained from a second source.

6.5.4 The matrix spike sample may be used as an LCS when the matrix spike acceptance criteria are as stringent as the LCS acceptance criteria and a LCS has not been prepared and analyzed for the sample batch.

6.5.5 Each analytical SOP references quality control criteria to use in determining discrepancies and accepting data when laboratory control samples do not meet acceptance criteria.

6.5.6 Section 15, Corrective Action Policies and Procedures, references procedures for taking corrective actions when laboratory control samples do not meet acceptance criteria.

6.6 Matrix Spike Duplicates and Sample Duplicates

6.6.1 Matrix spike duplicates or sample duplicates are performed at a rate of one per 20 or fewer environmental samples per matrix type, per sample extraction, or preparation procedure.

6.6.2 The sample used for the matrix spike duplicate is the same as used for the matrix spike.

6.6.3 When sample duplicate analyses are performed, samples are selected on a rotating basis from among various water samples, wastewater samples, monitoring locations, and other applicable locations.

6.6.4 All procedures used to select samples for matrix spike duplicate or sample duplicate analyses are documented.

6.6.5 Each analytical SOP references quality control criteria to use in determining discrepancies and accepting data when duplicate samples do not meet acceptance criteria.

6.6.6 Section 15, Corrective Action Policies and Procedures, references procedures for taking corrective actions when duplicate samples do not meet acceptance criteria.

6.7 Quality Control Charts and Tabulations

6.7.1 The laboratory maintains tabulations, quality control charts, or a combination of tabulations and quality control charts for the results from all quality control procedures. All calculations for the tabulations and/or control charts are performed according to *Standard Methods, 21th Edition,* Part 1020-B.

6.7.2 Separate tabulations and/or control charts are maintained for each test method, for each matrix, and for each analytical range.

6.7.3 Each tabulation and/or control chart includes the following information:

- Title
- Identification of standard operating procedure
- Name of quality control procedure being tabulated
- Analytical method
- Analyte
- Analyte units of measure
- Matrix
- Fortification concentration

- Mean
- Standard Deviation
- Upper Control Limit (UCL)
- Lower Control Limit (LCL)
- Upper Warning Limit (UWL)
- Lower Warning Limit (LWL)
- Date of Analysis
- Analyst Identification

6.8 Initial Demonstration of Method Performance Studies (IDMP)

6.8.1 Each analyst performs an IDMP study prior to initiation of sample analyses, unless the IDMP is not applicable to the approved test method, such as: BOD, CBOD, total suspended solids, total dissolved solids, total volatile solids, total solids, pH, temperature, dissolved oxygen, or turbidity. The IDMP study is repeated whenever there is a change in analyst, instrument type, or approved test method. The following steps are performed for an IDMP study:

- A quality control (QC) check sample is obtained from an outside source. If not available, the QC check sample may be prepared by the laboratory using standards that are prepared separately from the calibration standards by someone not running the test.

- The laboratory prepares four aliquots of the QC check samples at the required method volume to a concentration approximately 10 times the method-stated or laboratory-calculated MDL.

- The three aliquots are prepared and analyzed according to the approved test method.

- Using the three results, the average recovery and standard deviation are calculated in the appropriate units for each analyte.

- For each analyte, the standard deviation and average recovery are compared to the corresponding acceptance criteria for precision and accuracy in the approved test method (if applicable) or laboratory-generated acceptance criteria (if a non-standard method). If standard deviation and average recovery for all analytes meet the acceptance criteria, the analysis of actual samples may begin. If any one of the analytes exceeds the acceptance range, the performance is considered unacceptable for that analyte.

6.8.2 The laboratory management maintains a file to track all current IDMP studies.

6.9 Method Detection Limits (MDL)

6.9.1 MDLs for each analyte of interest are determined by the test method procedure specified in 40 CFR, Part 136, Appendix B, unless the test method specifies another procedure for MDL determination, or the determination of an MDL is not applicable to the test method.

6.9.2 The laboratory analyzes a minimum of seven replicates to determine the MDL

- If seven replicates are analyzed, the laboratory uses all analytical results when calculating the MDL.
- If the laboratory analyzes more than seven replicates, the laboratory only excludes analytical results which the laboratory determines are outliers by utilizing a statistical outlier test.

6.9.3 The laboratory determines MDLs for each approved test method annually; and when there is a change in instrument type.

6.9.4 The laboratory may, in lieu of the annual determination of the MDL, annually verify the MDL by the preparation and analysis of a minimum of one matrix spike sample, spiked at the current MDL. Results are verified by the laboratory supervisor.

- An MDL is considered verified and acceptable for continued use if results of the analysis of the matrix spike sample are within the 95% confidence interval as set forth in Appendix B of 40 CFR, Part 136.
- If an MDL cannot be verified, a new MDL is determined.

6.9.5 MDL replicate percent a recovery acceptance criterion is defined by the range of the percent mean recovery ± 2 times the percent relative standard deviation (% RSD) found for the seven replicates. If any of the seven replicates fails this acceptance criterion, then the analyst discards all results and performs another set of seven replicates.

6.9.6 The spiking concentrations used to determine an MDL are between 1 and 10 times the calculated MDL.

6.10 Internal Audits

6.10.1 The laboratory conducts annual internal audits of its operations, QA/QC practices, and record keeping.

6.10.2 The internal audit is performed by the designated quality assurance officer.

6.10.3 The results of the internal audit should specify procedures or practices that are not in compliance with the QAP, and corrective action shall be taken and documented.

6.10.4 Where the results of an internal audit indicate that the laboratory's test results are invalid, the laboratory takes immediate corrective action and immediately notifies, in writing, the parties that receive the data.

6.11 Analytical Quality Control Standards

All analytical quality control standards that are used are traceable to a National Standard.

6.12 Reagents

Reagents are prepared using reagent grade chemicals or better. Each reagent container received by the laboratory is documented by marking the container with an adhesive label and indelible ink. Each marking label indicates the date received, date opened, and any applicable expiration date.

6.13 Glassware

Glassware used for purposes that may subject it to damage from heat or chemicals is made of borosilicate glass. Glassware used for volumetric measurement purposes is Class A rated.

6.14 Laboratory Pure Water

The Town Branch Laboratory uses Nanopure grade water. Tap water is purified through a series of steps including: coarse filtration, water softening, chlorine removal filtration, reverse osmosis purification, and 4-cartridge Nano-pure purification process. The nano-pure system provides continuous conductivity readings of the of the final product. Conductivity values range between 17.5 and 18.0 megOhm. Readings below this range indicate spent cartridges and a need to service the unit. In addition, the resistivity of the Nanopure water is checked periodically with a calibrated conductivity meter. Resistivity values of the laboratory pure water are at least 0.5 megOhm/cm at 25°C. Filters are replaced when the values reach 1.0 megOhm/cm at 25°C.

Section 7
Verification Practices

7.1 Performance Evaluation (PE) Testing

7.1.1 Samples are analyzed for this laboratory facility as appropriate for the mandatory USEPA DMR-QA Laboratory Performance Evaluation Study.

7.1.2 PE samples are analyzed once per year, where appropriate samples are available, for each test method, each matrix and each analyte included in its scope of accreditation as required by the provisions in the DMRQA Announcement Letter (308 Letter) as well as the requirements detailed in the U.S. EPA National Standards for Water Proficiency Testing Studies Criteria Document (December 30, 1998).

7.1.3 PE samples are processed without any extraordinary care as the results obtained will be considered typical of the laboratory's performance. PE samples are treated as "unknown samples" according to guidelines described in Section 11. All directions are followed without changing sample preparation, dilution, or analysis.

7.1.4 The laboratory's personnel do not engage in inter-laboratory communications regarding PE sample results, or attempt to obtain the true values of the PE samples prior to reporting at the designated deadline for the PE Study.

7.1.5 All unacceptable results for PE samples are investigated by a standardized procedure. Appropriate corrective actions are implemented where assignable error was found. When assignable error is not found, corrective actions are focused on review of the procedure and improvement of test method execution. The test procedure is re-validated by successful analysis of a second source standard or reference material.

7.2 Standard Reference Materials

The laboratory uses standards that are traceable to Standard Reference Materials (SRMs), where available.

7.3 Internal Quality Control Programs

7.4.1 Statistical Process Control is generally used to establish batch acceptance criteria for analytical test results. The test results for the laboratory control standard are evaluated annually to set limits for control charts. Warning limits are set at $\pm\ 2\sigma$ from the mean recovery, and control limits are set at $\pm\ 3\sigma$.

7.4.2 When a control limit is exceeded, the analyst is required to respond in a manner that assumes something is wrong with the measurement system. Whenever possible, a response to an exceeded control limit includes the following:

- Inform the immediate supervisor of the control limit exceedance.
- Stop the analysis of samples, if possible.
- Conduct a systematic investigation as soon as possible to locate the source of the problem.
- Take appropriate corrective action when a problem is located.
- Rerun samples to the last good laboratory control standard whenever possible.
- Document the control limit event, including the details of the occurrence, whether a problem was detected, and any corrective actions taken.
- Maintain a state of increased vigilance.

7.4.3 Warning limit trend exceedance occurs when two or more consecutive results for the laboratory control standard exceed the warning limit. A general response to a trend exceedance includes the following.

- Inform the immediate supervisor of the warning limit trend exceedance.
- Conduct a systematic investigation as soon as possible to locate the source of the problem.
- Take appropriate corrective action when a problem is located.
- Document warning limit exceedance, including the details of the occurrence, whether a problem was detected, and any corrective actions taken.

7.4.4 The laboratory has a written response plan for the analyst to follow in the event of a failure of a laboratory control standard or other criteria. This plan is found in Section 15, Corrective Action Policies and Procedures.

7.4.5 SOPs for test methods and lab procedures are reviewed on an annual basis. The designated Quality Assurance Officer and Laboratory Supervisor performs reviews.

Section 8
Equipment Procedures for Calibration, Verifications, and Maintenance

8.1 Equipment Calibration

8.1.1 An initial calibration is performed on instrumentation and equipment as specified in the test method. Calibration standards are traceable to a National Standard, where available.

8.1.2 The procedures for calibration verification and maintenance are found in the analytical method SOPs or sample preparation SOPs. Manufacturers operation manuals may be referenced in the method SOPs when they are the source for calibration or maintenance procedures.

8.1.3 Documentation is maintained for all maintenance, calibration and instrument operation activities. All defective equipment is removed from service and is not returned to operation until repaired and shown by calibration, certification or test to perform satisfactorily.

8.1.4 An adequate number of standards are used to define the calibration curve. The test method SOP states if the calibration curve is linear or non-linear.

8.1.5 If the test method does not state the number of calibration standards to use, the laboratory will use a minimum of 7 concentrations to create the calibration curve. The range should be within the linear range of the curve, and should correspond to values typical for the samples.

8.1.6 Unless specified by the test method, the lowest calibration standard is set at 1 to 15 times the MDL whenever sample results will be used in a decision related to the determination of a non-occurrence of an analyte or a non-detect the MDL of an analyte.

8.1.7 All sample results for test methods utilizing a calibration curve are reported within the highest calibration standard, or within the linear dynamic range where the test method requires determination of the linear dynamic range.

8.1.8 Further guidance regarding calibration may be found in Section 186.155 of Illinois regulations Title 35: Environmental Protection, Subtitle A: General Provisions, Chapter II: Environmental Protection Agency, Part 186: Accreditation of Laboratories for Drinking Water, Wastewater, and Hazardous Waste Analysis. Copies of this document may be obtained from the laboratory supervisor or the quality assurance officer.

8.2 Balances

The laboratory's analytical balance has a sensitivity of 0.1 mg. All analytical balances are placed on a stabilizing slab base. Each balance is checked daily with two or more ASTM type 1 weights, which cover the effective range of the balance's use. All balances are serviced and calibrated at least annually by a qualified service representative. The service representative issues the laboratory a certificate of calibration with weights traceable to National Standards.

8.3 pH Meters

All pH meters have an accuracy of at least ± 0.1 pH units and provide for temperature correction of pH measurements. Daily calibrations are performed with a minimum of three standardization buffers as specified in SOP-pH. The pH of the standardization buffers are 4.0, 7.0 and 10.0.

8.4 Conductivity Meters

A conductivity meter is maintained with an error not exceeding 1% or one micromhos/cm, whichever is greater. The conductivity meter is calibrated before each use with a standard that reflects the sample conductivity.

8.5 Thermometers

8.5.1 The laboratory has certified thermometers traceable to National Standards, with 1 degree centigrade or finer subdivisions and a range which spans the various requirements of the analytical methods, equipment temperature monitoring, and checking for thermal preservation. These traceable thermometers are recalibrated a minimum of every five years. The laboratory maintains Certificates of Calibration that identify traceability of the calibration to National Standards.

8.5.2 All other thermometers are calibrated against thermometers traceable to National Standards. Liquid-in-glass and digital thermometers are calibrated annually. Metal and continuously monitoring thermometers are calibrated at least quarterly. Calibration factors are employed based upon the most recent calibration.

8.6 Refrigerators

8.6.1 Each refrigerator is uniquely identified and provided with a uniquely identified thermometer graduated in increments no larger than 1 degree centigrade. Thermometer readings are monitored and recorded each day the laboratory is in operation. The monitoring logs include refrigerator and thermometer identification, date, time, temperature, initials of the responsible person, and the acceptable temperature range.

8.6.2 The following table lists the refrigerators that are used in the Town Branch Laboratory.

Description	Unique Identifier	Range
Room Temperature Storage Unit	Room Temp Storage Unit	10 - 30° C
Microbiology Station	Refrigerator #1	4.0 ± 2.0° C
Pretreatment Sample Storage	Refrigerator #2	4.0 ± 2.0° C
Town Branch Sample Storage	Refrigerator #3	4.0 ± 2.0° C
West Hickman Sample Storage	Refrigerator #4	4.0 ± 2.0° C
Chemical Storage	Refrigerator #6	4.0 ± 2.0° C

8.6.3 Samples which require thermal preservation are stored under refrigeration which is ± 2° C of the specified preservation temperature, unless method specific criteria exist. For samples with a specified storage temperature of 4° C, storage temperatures of 4.0 ± 2.0° C are acceptable.

8.7 Incubators

8.7.1 Each incubator is uniquely identified and provided with a uniquely identified thermometer graduated in increments no larger than 1° C. Thermometer readings are monitored and recorded each day the laboratory is in operation. The monitoring logs include incubator and thermometer identification, date, time, temperature, initials of the responsible person, and the acceptable temperature range.

8.7.2 The following table lists the incubators that are used in the Town Branch Laboratory.

Description	Unique Identifier	Range
BOD Incubator	BOD Incubator #1	20.0 ± 1.0° C
BOD Incubator	BOD Incubator #2	20.0 ± 1.0° C
Coliform Incubator Bath	Precision #1	44.5 ± 0.2° C
Coliform Incubator Bath	Precision #2	44.5 ± 0.2° C
Coliform Incubator Bath	Precision #3	35.0 ± 0.2° C

8.8 Ovens

8.8.1 Each oven is uniquely identified and provided with a uniquely identified thermometer, graduated in increments no larger than 10° C for muffle furnaces and 1° C increments for oven and warm air incubators. Temperatures are recorded daily. The monitoring logs include; oven and thermometer identification, date, time, temperature, initials of the responsible person, and the acceptable temperature range.

8.8.2 The following table lists the ovens that are used in the Town Branch Laboratory.

Description	Unique Identifier	Comments
Drying Oven	Drying Oven #1	103 – 105^{o} C
Iso-temperature Oven	Iso Temp Oven	Adjustable
Muffle Furnace	Muffle Furnace	Adjustable

8.9 Other Laboratory Equipment

8.9.1 The laboratory monitors and controls method specific temperature requirements for heating blocks and water baths, etc. The laboratory also maintains documentation of the results.

8.9.2 Autopipetors and dilutors of sufficient accuracy are used for some applications. The autopipetors (repipettors) are serviced and calibrated annually by a qualified service representative. The service representative issues the laboratory a certificate of calibration for each unit and labels each unit to indicate service/calibration. Logs of these checks are maintained for each device.

Section 9
Test Methods and SOPs

9.1 Scope

The Standard Operating Procedures section describes all procedures currently in use by the laboratory. Included in this section are each method's SOP number, document name, source, and whether it is listed in 40 CFR Part 136 as an approved method.

9.2 Standard Operating Procedures (SOPs)

The SOPs are incorporated in this Quality Assurance Plan and may be obtained from the laboratory management. In addition, each laboratory staff member is issued a manual containing all current SOPs. A laboratory copy is used for updates and changes between revisions.

Section 10
The Laboratory's Physical Facilities Including Services and Resources

10.1 Building

The Town Branch Laboratory, operated under the Lexington-Fayette Urban County Government, is located in the Town Branch Administration building, 301 Lisle Industrial Avenue, Lexington, Kentucky. The Town Branch Laboratory performs routine monitoring for Town Branch Wastewater Treatment Plant, West Hickman WWTP, and Blue Sky WWTP, several industrial pretreatment samples, and samples brought in from other outside sources. The Administration building housing the laboratory was constructed in 1970. Currently, the laboratory and offices occupy 5,735 square feet of floor space. The laboratory originally occupied 2,370 square feet of floor space. In 2004, the lab was expanded to include an additional 3,365 square feet of floor space. The new addition includes additional bench space and cabinets. A schematic layout of the laboratory showing current space utilization is shown in Appendix 5.

10.2 Access and Security Measures

10.2.1 Entrances to the Town Brach WWTP are located at the north-east and south ends of the plant. Automatic gates are open during normal operating hours. Magnetic access cards are required for entry during off hours.

10.2.2 Any visitors to the Town Branch WWTP and Laboratory are required to sign in with Reception in the Administrative Building.

10.2.3 Normal operating hours for the laboratory are 7:30 AM to 4:00 PM, Monday through Friday. A Laboratory Technician is present 7:30 AM to 4:00 PM on Saturdays.

10.3 Building Services

10.3.1 Air Temperature and Quality - The laboratory's heating and air-conditioning are generally to control laboratory temperatures between 68 and 75^{o} F and temperature fluctuations rarely exceed more than 4 degrees per hour.

10.3.2 Electricity - 110 and 220 volt electrical service is provided throughout the laboratory.

10.3.3 Illumination - Lighting in most areas and all work areas is provided by fluorescent lighting. Emergency lighting is located throughout the lab.

10.4 Safety

10.4.1 Fire Safety – Fire alarms are located throughout the building. Fire extinguishers are prominently displayed throughout the lab and all laboratory personnel are trained yearly in the use and safe handling of the extinguishers.

10.4.2 Other Safety Equipment – The Town Branch lab maintains a fully stocked first aid kit, two eye-wash and shower stations. All safety equipment is inspected on a monthly basis. The lab has a total of 8 chemical hoods, which are inspected and serviced yearly. Chemical neutralization and spill control kits, and acid and flammable storage cabinets are located in the chemical storage room. Current MSDS sheets are maintained for all chemicals used in the lab.

10.5 Computer Resources Including Equipment and Software

The LFUCG IT department is responsible for all computers and software used in the laboratory. A total of 5 PC computers are located throughout the laboratory. All computers have access to the internet via the City's server. Password protections restricts access to lab data to lab personnel only. All data is viewable as a "read-only" file. In addition to the Excel-based database, all laboratory computers can access the HACH Water Information Management Solution (WIMS) database which houses all laboratory information.

10.6 Laboratory Work Areas

Several sections in the laboratory have been reserved for specific analyses. These sections include: sample receiving, microbiology, BOD preparation/analysis, wet-chemistry, total residual chlorine analysis, total cyanide digestion, solids processing/weighing, spectrophotometric methods (i.e. HACH TNT+ methods), water purification. Employees are routinely rotated through the different procedures.

Section 11
Procedures for Reviewing New Work

11.1 Scope

Laboratory management reviews all new work to ensure the laboratory is able to perform the additional task(s) in a timely and accurate manner. Laboratory management consults, if necessary, with sampling personnel and end data users to ensure sample integrity and data quality.

11.2 Unknown Samples

On occasions, the Laboratory will receive and analyze samples from industrial pretreatment sources or samples brought in from other outside sources. These samples are considered "unknown samples" for which duplicate sub-samples are processed and analyzed. All QA/QC requirements apply to the unknown samples.

11.3 Contract Analyses

The laboratory does not perform contract analyses.

Section 12
Sample Acceptance and Receipt Policy

12.1 Scope

Sampling is critical to producing data representative of conditions that occur at the sampling location. This section of the QAP constitutes the laboratory's written sample acceptance policy. It details the conditions under which the laboratory will accept samples. It is readily available to all laboratory staff and sample collectors.

12.2 Sample Acceptance Requirements

12.2.1 The Plant Operator confirms the appropriate sampling plan in order to verify that sampling techniques are consistent with the intended use of the data. Sampling equipment is selected and pre-cleaned to preserve sample integrity and eliminate contamination.

12.2.2 Accepted samples are logged upon arrival and checked for sampling preservation and holding times.

12.2.3 Indelible ink is used for all written documentation associated with samples. Corrections to sampling documents are made by a single line strikeout. The corrected entry is written above the strikeout and initialed by the person making the correction.

12.2.4 Samples must be received with complete documentation in the form of a Chain of Custody (COC) record including sample identification, location, date and time of collection, collector's name, sample type, person receiving sample, and any special remarks.

12.2.5 **COC Records**
The different COC records used at Town Branch Laboratory are:

1) Town Branch 24-hour composite samples with COC record (delivered to the Town Branch Lab by the night Operator at midnight the night before).

2) Town Branch grab samples with COC record (delivered to the Town Branch Lab each morning by the on-duty Operator).

3) Town Branch solids grab samples and composite samples with COC record (delivered to the Town Branch Lab each morning by the Solids Operator).

4) West Hickman grab and composite samples are on one COC record (delivered to the Town Branch Lab each morning by the West Hickman on-duty Operator).

5) Blue Sky grab and composite samples are on one COC record (delivered to Town Branch Lab every Thursday by the Blue Sky on-duty Operator).

6) Illicit Discharge Detection and Elimination (IDDE) grab samples with COC record (delivered to Town Branch lab by the Environmental Inspector).

7) Industrial waste 24-hour composite samples COC record (delivered to the Town Branch Lab by the Environmental Inspector).

12.2.6 COC Checklist

1) Operator obtains COC record.

2) Operator collects samples.

3) On COC record operator records:
   - date sampling started
   - time sampling started
   - date sampling stopped
   - time sampling stopped
   - sampling method
   - sample type
   - sample size
   - type of preservative
   - analysis requested
   - sampler's signature

4) Operator delivers samples to Town Branch Lab.

5) Operator signs the COC sheet on the line "*Relinquished By (Representing Operators) (Signature)*".

6) Operator writes the date and time of relinquishment on the same line as noted above in (5).

7) Operator leaves the COC sheet with a Laboratory Analyst who then signs the sheet on the line "*Received in the Laboratory By (Signature)*".
   - On the same line the Laboratory Analyst writes the date and time the samples were received in the lab.

12.2.7 Samples must be received in the sample container required by the test method and properly labeled.

12.2.8 Each sample container must have a unique identification.

12.2.9 Sample preservation must comply with the requirements of the approved test method.

12.2.10 Sample volume must be sufficient to perform the necessary analyses.

12.2.11 Samples must be received within the time specified by the test method. Upon receiving a sample, the laboratory will determine if the analysis can be performed within the allotted holding time.

12.2.12 Bypass samples and Town Branch 24-hour composite samples are collected the night before analysis and are delivered to Town Branch Laboratory and kept in a secure location until analysis. The chain of custody (COC) form is stored with the samples.

12.2.13 Town Branch grab samples, West Hickman 24-hour composite samples, Blue Sky 24-hour composite samples, and Industrial Waste 24-hour composite samples are brought to the Laboratory Analyst during operating hours.

12.3 Verification of Preservation

12.3.1 Samples are examined for method preservation and proper documentation.

12.3.2 During 24 hour composition, samples are examined daily for thermal preservation. Thermal preservation will be acceptable if:

- Temperature is either within ± 2° C or at the method's specified range. For samples with a specified temperature of 4° C, a temperature of 2.0 to 6.0° C is acceptable.

- Samples have been hand delivered to the laboratory within 6 hours of collection and there is evidence that the chilling process has begun, such as arrival on ice.

12.3.3 Town Branch Lab refrigerated storage unit temperatures are checked and documented daily. Temperature must be 4º C ± 2° C.

12.3.4 Samples are examined for chemical preservation upon receipt, or prior to additional sample preparation or analysis.

12.4 Actions for Deviations from the Lab's Sample Acceptance Requirements

12.4.1 Deviations from the sample acceptance policy is brought to the attention of the Laboratory Analyst and documented at the time of sample log-in.

12.4.2 Whenever possible, the laboratory proceeds to correct errors in sample documentation. The person responsible for the error is contacted so that a deviation from the sample acceptance policy can be avoided.

12.4.3 If a sample does not meet all of the sample acceptance criteria:

- Retain correspondence and records of information concerning the final disposition of rejected samples.
- Contact client from which the laboratory received samples.

12.5 Laboratory Log of Received Samples

Each sample received is logged with the following information:

- date and time of laboratory receipt of sample
- sample collection date
- whether sample is composite or grab
- unique laboratory identification code
- sample collection point
- requested analyses
- signature or initials of data logger
- comments resulting from inspection for acceptance or rejection
- sample collector
- person receiving sample

Section 13
Sample Tracking and Storage Procedures

13.1 Scope

A clear sample tracking record allows any questions concerning sample integrity to be answered during any step of the process. Samples are stored in areas which isolate samples sufficiently to prevent cross-contamination with other samples, reagents or standards. Procedures for sample handling and tracking may vary, depending upon sample origin. Sample integrity and reliable test data are ensured when valid laboratory procedures are established and strictly followed.

13.2 Sample Tracking Record

13.2.1 Sample logs, bench sheets, and preparatory records are clearly marked to identify all personnel associated with each sample.

13.2.2 Chain of custody forms or bench sheets must be completed to include use of preservation and sample containers required by approved test method.

13.2.3 No samples are accepted without proper documentation. Samples are checked to insure that the container is compatible with the intended analysis.

13.3 Proper Storage and Avoidance of Cross-contamination of Samples

13.3.1 Storage facilities are provided for samples to prevent cross-contamination and are consistent with sample preservation requirements of the method.

13.3.2 Samples are stored away from all standards, reagents, food and other potentially contaminating sources.

13.4 Security of Samples

The laboratory stores all samples within the confines of the laboratory and limits access to authorized laboratory personnel only.

13.5 Sample Disposal

If possible, samples and aliquots are kept to the end of the maximum permitted holding time in the event that re-analysis of the sample is required. Proper temperature and holding times must be observed for re-analysis. Analyzed samples or samples with expired holding times are disposed of properly.

Section 14
Record Keeping, Data Review and Reporting Procedures

14.1 Scope

The record keeping system allows historical reconstruction of all laboratory activities that produce the resultant sample analytical data. The laboratory maintains complete sample tracking records. Documentation of laboratory activities includes inter-laboratory transfers of samples and sample extracts. The laboratory issues sample data or sample result reports accurately and in a manner that is understandable to the recipient.

14.2 Records Maintained by the Laboratory

14.2.1 The laboratory retains records related to all procedures and activities to which a sample is subjected. These records include:

1) Identity of personnel involved in sampling, preparation and testing.

2) Sample preservation, including sample container and compliance with holding times.

3) Sample identification number, receipt, log-in, acceptance or rejection.

4) Sample storage and tracking, including shipping receipts, transmittal forms, and internal routing and assignment records.

5) Sample preparation including: cleanup and separation procedures, sample identification codes, volumes, weights, instrument printouts, and calculations.

6) Sample analysis.

7) Equipment receipt, use, specification, operating conditions and preventative maintenance.

8) Calculations and statistical formulas used by the laboratory, including written procedures for calculations, raw data and supporting information used for each calculation, correct use of significant figures, and identification of the least precise step in accord with limitations of the measurement system.

9) Procedure to verify that the reported data is free from transcription and calculation errors.

10) Data handling, including reduction, review confirmation, interpretation, assessment or validation, and reporting.

11) QC measurements, including procedure to select samples on which to perform QC measurements, and assessment of method performance.

14.2.2 Other records retained by the laboratory include:

1) All original raw data, whether hard copy of electronic, for calibrations, analyst's worksheets, and data output from instruments or equipment.
2) Copies of final reports.
3) Archived SOPs.
4) Correspondence with sample submitters.
5) Corrective action reports, audits and audit responses.
6) PE sample results and raw data.

14.3 Records of Standards and Analytical Reagents

14.3.1 Traceability of standards - The laboratory verifies that standards are traceable to National Standards. If traceability is not possible, the laboratory demonstrates, by appropriate means (i.e., analyses of PE samples) that the instrumentation and equipment is properly calibrated.

14.3.2 Receipt and use of reagents and standards - The laboratory retains records of the origin, purity and traceability of all reagents and standards. These records also include the date of receipt, storage conditions, the date of opening and an expiration date.

14.3.3 Traceability of working and intermediate standards - The laboratory maintains records of traceability from working and intermediate standards to purchased stock standards or neat compounds which include the date or preparation and preparer's initials.

14.3.4 Identification of prepared reagents and standards - All prepared reagents and standards are labeled with the identity the reagent or standard, concentration, preparation date, and preparer's initials.

14.3.5 Records of Instrument or Equipment Calibrations

14.3.6 The laboratory documents and maintains calibration procedures that establish calibration frequency and calibration acceptance criteria.

14.3.7 All graphs of calibration curves have descriptive titles, labeled axes, and date of calibration, time of calibration, test method, analytic, standard concentrations, instrument response and the calibration curve.

14.3.8 All calibrations record the equation of the calibration curve and the correlation coefficient.

14.4 Computerized Data Storage

14.4.1 The laboratory establishes and implements procedures for protecting the integrity of the data, including data protection procedures during data entry and capture, data storage, data transmission and data processing. Data is protected with lab password access only.

14.4.2 The laboratory provides procedures for the maintenance of the security of the data, including the prevention of unauthorized access to data, and the unauthorized change of computer records.

14.4.3 The laboratory maintains hard copy and/or writes protected backup copies of computer generated records.

14.5 Administrative Records

14.5.1 Personnel qualifications - The laboratory maintains records of personnel qualifications, education, experience and training.

14.5.2 IDMP studies - The laboratory maintains records of IDMP studies and any required repetitions of the IDMP for each analyst.

14.5.3 Initials and signatures - The laboratory maintains a log of names, initials and signatures for all individuals who are responsible for signing or initialing any laboratory record.

14.6 Laboratory Record Entries and Change of Entries

14.6.1 Laboratory personnel sign or initial (all three initials) all record entries. The reason for the signature or initials is clearly indicated in the records, including, but not limited to: sampled by, prepared by, and reviewed by.

14.6.2 Permanent ink is used for manually recorded data.

14.6.3 Corrections of manually recorded data are made by one line through the error. The correction is initialed and dated. Corrections are not made by erasure or White Out™.

14.6.4 Electronically maintained records are kept in such a fashion as to indicate any change in the record.

14.6.5 After the laboratory delivers its sample data and results to client, the laboratory will only correct, add or delete information from the report when it supports those actions by supplementary documentation. Any supplemental report clearly identifies there purpose and contains all reporting requirements.

14.6.6 Facilities with in-house laboratories which provide data to regulatory agencies must include all items in Section 14.10 with their reports if so required by the regulatory agency.

14.7 Record Retention

14.7.1 All records are retained for a minimum of five years. They include information pertaining to environmental analyses, performance testing, obsolete or replaced procedures, and supplies for tests, support services, and laboratory accreditation.

14.7.2 Access to archived records is documented with an access log. All records are protected against loss of deterioration including fire, theft, and electromagnetic deterioration in the case of electronic records.

14.8 Raw Data Associated with Sample Analysis

All raw data associated with sample analyses (i.e., calibration curves, strip charts, tabular printouts, computer data files, analytical notebooks, and run logs) include the following:

1) Laboratory sample number.
2) Date of analysis.
3) Type of analysis.
4) Instrument identification.
5) Instrument operating conditions (or reference to such information).
6) All calculations automated or manual to which the sample data is subjected.
7) Analysts' and/or technician's initials or signature.

14.9 Test Data Reports

Laboratory sample data or sample result reports include the following:

1) Report title such as "laboratory results".
2) A unique identification of the report such as serial number.
3) Description and identification of samples.
4) Date of sample receipt, sample collection and sample analysis (time of sample preparation and analysis if the required holding time for either activity is less than or equal to 48 hours).
5) Characterization and condition of sample, where appropriate.
6) Reference to sampling procedure, where applicable.
7) Test method utilized.
8) Sample results with any failures or deviations from approved test methods or QC criteria identified, such as data qualifiers.
9) Description of the calculations or operations performed on the data, a summary and analysis of the data.
10) Identification of the reporting units (such as mg/L or mg/kg).

11) Identification of any results not generated by the laboratory preparing the data report and identification of the laboratory from which such results were obtained.
12) A statement that the report shall not be reproduced, except in full, without the written approval of the laboratory.
13) A statement that samples results relate only to the analytes of interest tested or to the sample as received by the laboratory.
14) Additional information may be required for sample data reports submitted to a regulatory authority.

The laboratory certifies that the sample results meet all the requirements of any environmental laboratory program for which it maintains accreditation.

14.10 Electronic Data Transfer

Electronic data transfer (i.e. fax, email attachments, CD, flash drives) are documented by hard copies.

14.11 Sample Disposal Records

Ordinarily, the laboratory does not need to maintain records for disposal of samples. For a record of possible litigation samples and other samples that the laboratory supervisor wishes to retain, such records will be catalogued by date of disposal and person responsible. Samples may be disposed of by sample depletion, sample returned to submitter, sample washed down sink, and sample manifested to a hazardous waste facility. Any correspondence concerning sample disposal must be retained. Additional information on confidentiality is presented in Section 18.

14.12 Waste Disposal

Waste collection, storage, recycling, and disposal procedures and policies are part of method SOPs. Where disposal procedures and policies are included as part of a test method, the test method disposal practices are strictly followed.

Section 15
Corrective Action Policies and Procedures

15.1 Scope

The following policies and procedures are used when any analysis or reporting discrepancies are detected, or when any deviation from the policies and procedures in this manual occur.

15.2 Identification of Discrepancies

15.2.1 Discrepancies or deviations shall be defined as, but not limited to, any of the following:

1) quality control sample results outside control limits
2) reporting sample results in wrong units
3) using unapproved analytical procedures
4) data which appears to be erroneous to current trends

15.2.2 Each analytical procedure SOP references quality control criteria to use in determining discrepancies and accepting data.

15.3 Staff Responsible for Investigation and Corrective Action

15.3.1 Any of the Laboratory staff may detect discrepancies. Once a discrepancy is detected, it is reported to laboratory supervisor. Laboratory management or staff may investigate any discrepancies. Data that has discrepancies should not be recorded and should be reviewed.

15.3.2 Once a discrepancy is established, staff should proceed with one or more of the following:

1) re-run samples, if available and holding time permits
2) document results as invalid and note on any reports
3) investigate discrepancy; document cause and corrective action taken and include with data

15.3.3 Staff responsible for investigating the discrepancy shall review sample and quality control results, integrity of quality control samples, and technique. If a quality control result does not meet method or laboratory criteria, it shall be documented on the analysis bench sheet and quality control chart. Staff should also review sampling procedures and preservation, washing of glassware, and any sources of contamination to samples or quality control standards.

15.3.4 The Laboratory staff performs corrective actions to eliminate any of the above mentioned possible causes for data discrepancies. Examples of corrective actions are: review of proper procedures, quality control standard preparation, changes to procedures or sampling protocol, and improving analyst technique. If the investigation cannot determine a known cause for invalid results, retraining and procedure review are the appropriate corrective actions.

15.3.4 For each data discrepancy event, the investigation and corrective action shall be documented.

15.4 Documentation and Review of Corrective Action

Laboratory management reviews raw data, reports, quality control data, any discrepancies, and corrective actions on a regular basis. This review helps establish any recurring problems that require further investigation and action.

Section 16
Procedures for Permitting Departures from Documented Policies and Procedures

16.1 Scope

Departures from documented policies and procedures may include, but not limited to:

- use of analytical methodology which is not KYDEP or U.S. EPA approved
- use of samples which are not properly sampled or preserved for intended purpose
- analysis of samples outside the required holding times
- deviations from analytical or other laboratory SOPs
- deviations from standard QC practices
- use of alternative calibration practices
- reporting data which is not compliant with KYDEP or U.S. EPA analytical or QC requirements

16.2 Requests for and Approval of Departures

Requests for allowing departure from documented policies and procedures are directed to the Laboratory Supervisor. The Laboratory Supervisor shall evaluate the reasons for such departures. The Quality Assurance officer is consulted for all matters that may affect the quality of analytical data. Written approval by the Laboratory Supervisor is required before such departures are made. The Laboratory Supervisor documents and files all departures and their duration. Permanent departures are documented by changes in the established policies and procedures.

16.3 Use of Analytical Methods Not Approved by KYDEP or U.S. EPA

When analytical methods are used which are not approved by KYDEP or U.S. EPA, SOPs are used to document the actual procedures performed as well as the source(s) of the method. The methods used to determine the criteria for IDMP are established and documented prior to use of the method. Quality assurance and control procedures are included in the method.

16.4 Reporting of Non-compliant Data

Data which is produced from samples that do not meet the regulatory sampling, holding, or preservation requirements, or which is produced using methods that deviate from EPA-required analytical or QC requirements, is reported only when accompanied by a statement that clearly indicates that the data may not be used for regulatory compliance purposes.

Section 17
Procedures for Dealing with Complaints

17.1 Scope

The Town Branch Laboratory has specific procedures for dealing with complaints from clients or other parties.

17.2 Complaint Receipt Procedures

17.2.1 Document the initial complaint with the following information:

- Name of person with complaint
- Company name
- Phone number
- Sample Identification
- Nature of complaint

17.2.2 Inform person with complaint that it will be investigated promptly. Also inform him of the estimated time or date for a response to the complaint.

17.3 Complaint Investigation Procedures

17.3.1 Review the following items regarding the sample or analysis in question with laboratory staff:

- Receipt of sample
- Internal Chain of Custody
- Analyses performed
- Analysis methods
- Analytical results
- Calibration check
- Quality control results
- Calculations
- Unit conversions

17.3.2 Document whether any discrepancies were revealed during the review, especially discrepancies which address specific complaint.

17.4 Complaint Response Procedures

17.4.1 Laboratory management will contact the person with complaint and reveal findings of investigation. If necessary, a corrected report will be issued.

17.4.2 File the documented complaint investigation for use during the annual review of the Quality Assurance Plan. If any corrective actions are required, reference Section 15 of this QAP manual, “Corrective Action Policies and Procedures.”

Section 18
Procedures for Protecting Confidentiality and Proprietary Rights

18.1 Procedure for Protecting Confidentiality

The Town Branch Laboratory must protect confidentiality and proprietary rights. Laboratory employees are instructed not to divulge any information that may involve issues of confidentiality or proprietary without the approval of the Laboratory Supervisor.

18.2 Examples of Confidential Data

Some examples of confidential data include:

- Effluent and/or Permit data.
- Analytical results for Plant operation samples.
- Analytical results for samples obtained from industrial dischargers.
- Results obtained for the PE.
- Sample results that may be integral to on-going or possible litigation.

18.3 Procedure for Open-Records Requests

Requests for open-records must be made in writing to the Laboratory Supervisor. The requests will be reviewed by the Laboratory Supervisor and require final authorization by the Division Director before release of records. The request and released records will be filed by the Laboratory Supervisor.

Section 19
Procedures for Internal Audits

19.1 Annual Internal Audit

The Quality Assurance Officer and/or Laboratory Supervisor conducts an annual internal audit of the laboratory.

19.1.1. This auditor conducts a systematic audit of technical activities from previously prepared checklists.

19.1.2. The auditor determines whether the quality assurance practices and other laboratory procedures described or referenced in the QAP have been implemented.

19.1.3. The auditor then prepares a written report which includes copies of the check-lists and a list of all noted deficiencies.

19.2 Corrective Actions Regarding the Annual Internal Audit

Laboratory management takes appropriate corrective actions in response to the internal audit, including a written response plan which covers:

- Completed corrective actions
- Planned corrective actions
- Implementation schedule for planned corrective actions.

19.3 Notification of Invalid Data

In the event that the results of the internal audit indicate that the laboratory's test results are invalid, immediate corrective action is taken. All persons are notified who received the invalid data.

Section 20
Annual Review of the Quality Assurance Plan (QAP)

20.1 Scope

The QAP is reviewed annually. The Laboratory Supervisor conducts an internal review with the Quality Assurance Officer. The laboratory, in its review, determines whether requirements of the QAP have been adequately addressed, the results of the annual review must be documented.

20.2 Correcting Deficiencies in the QAP

If the Laboratory Supervisor and Quality Assurance Officer find deficiencies, the QAP is revised as necessary. The revised QAP is then issued with the appropriate version number and implemented at the agreed upon effective date. All copies of the preceding version are replaced with the new version.

Section 21
Training and Personnel Requirements

21.1 Scope and Training Objectives

All personnel involved in laboratory analysis or quality assurance/quality control shall have sufficient training to allow for the analysis and reporting of complete, high quality data in compliance with the procedures of this Comprehensive Quality Assurance Plan. Laboratory Management is responsible for ensuring the required training is made available.

21.2 Training Required for Laboratory Supervisor

21.2.1 The Laboratory Supervisor shall hold a bachelor's degree in natural or physical sciences or have completed enough course work in chemistry to equal a major in chemistry.

21.2.2 The Laboratory Supervisor shall have a minimum of one year's experience in analyses pertaining to the applicable fields of testing.

21.3 Training Required for Quality Assurance Officer

21.3.1 The QA Officer shall hold a bachelor's degree in natural or physical sciences or have completed enough course work in chemistry to equal a major in chemistry.

21.3.2 The QA Officer shall have a minimum of one year's experience as an analyst in a laboratory.

21.3.3 The QA Officer shall have documented training in quality assurance and quality control.

21.4 Training Required for Technicians

21.4.1 Technicians shall hold a bachelor's degree in natural or physical sciences or have completed enough course work to equal a major in chemistry.

21.4.2 Technicians shall have a minimum of one year's experience in the analyses pertaining to the applicable fields of testing.

21.4.3 Technicians shall meet the instrument training requirements specified in Section 21.9.

21.4.4 After completing training, technicians shall perform an Initial Demonstration of Method Performance (IDMP) study for each analysis as specified in Section 6.8.

21.4.5 The laboratory shall have on file documentation indicating the analyst's acceptable performance on a blind sample at least once per year and a certification that the analyst has read, understood and agreed to perform the most recent version of the standard operating procedure.

21.5 Additional Training for Technicians

Additional training, provided by the Laboratory Management, involves participation in the Kentucky Laboratory Analyst (KLA) training, testing, and certification for laboratory technicians. The KLA was created under the KY Water and Wastewater Operators' Association (KWWOA) for individuals employed in the water quality field. A total of 4 class certifications are available for Wastewater Laboratory Analysts.

21.6 Substituting Experience or Education to Meet Training Requirements

A person may serve as Laboratory Supervisor, Quality Assurance Officer, or Technician when that person does not meet the training, educational or experience requirements for the position. In such cases the laboratory shall submit written justification explaining why a person should serve in a particular position. This written justification shall take into account the following factors:

- Experience as an offset for education requirements.
- Education as an offset for experience requirements.

21.7 Instrument Training Requirements

Analysts and technicians must meet either of the following requirements for analyses performed utilizing specialized laboratory instrumentation (i.e., Atomic Absorption):

- The technician shall have satisfactorily completed a minimum of four hours training that is offered by the equipment manufacturer, a professional organization, a university, or qualified training facility.
- The technician shall have served a two week period of apprenticeship under an experienced staff member.

21.7 Training Records

Laboratory management maintains employee training records. The records are updated periodically for each employee receiving training.

Section 22
Glossary

Acid - An inorganic or organic compound that (a) reacts with metals to yield hydrogen; (b) reacts with a base to form a salt; (c) dissociates in water to yield hydrogen or hydroniumions; (d) has a pH of less than 7.0; and (e) neutralizes bases or alkaline media.

Accuracy - Degree of conformity of a measure or test to a standard or true value.

Aliquot - A portion of a sample.

Alkali - Any compound having highly basic properties.

ATM - Atmosphere, pressure measurement.

Arsenic (As) - The major source of occupational exposure to arsenic is in the manufacture of pesticides, herbicides, and other agricultural products.

Barium (Ba) - Barium is used in various alloys, in paints, soap, paper and rubber.

Base - A substance that usually liberates OH anions. Bases have a pH greater than 7.00.

BOD - Biochemical Oxygen Demand is a measure of the quantity of oxygen utilized in the biochemical oxidation of organic matter related to the oxygen requirements in chemical combustion, being determined entirely by the availability of the material as a biological food and by the amount of oxygen utilized by the microorganisms during oxidation. BOD is the initial quantity of oxygen used by polluted liquid immediately upon being introduced into water containing dissolved oxygen. It may be exercised by end products of prior biochemical action or chemical substances avid for oxygen. The BOD content is usually expressed in pounds per unit of time, load of wastewater passing into a waste treatment system or to a body of water.

Biochemical Process - The process, by which, the metabolic activities of bacteria and other microorganisms break down complex organic materials into simple, more stable substances.

Biodegradation - The destruction of mineralization of either natural or synthetic organic materials by the microorganisms populating soils, natural bodies of water, or wastewater treatment systems.

Carcinogen - A material that either causes cancer to humans or animals.

Catalyst - A substance that modifies (slows or accelerates) a chemical reaction without being consumed.

Cadmium (Cd) - The main use of cadmium is in electroplating or galvanizing. It is also used as a color pigment for paints and plastics and cathode material for nickel-cadmium batteries.

COD - Chemical Oxygen Demand, a measure of the oxygen-consuming capacity of inorganic and organic matter present in water or wastewater. It is expressed as the amount of oxygen consumed from a chemical oxidant in a specific test. It does not differentiate between stable and unstable organic matter and thus does not necessarily correlate with biochemical oxygen demand. The method can be applied to domestic and industrial waste samples having an organic carbon concentration greater than 15 mg/L. For lower concentrations of carbon such as in surface water samples, the Low Level Modification should be used. Organic substances in the sample are oxidized by Potassium Dichromate in 50% Sulfuric Acid solution at reflux temperature. Silver Sulfate is used as a catalyst and Mercuric Sulfate is added to remove Chloride interference. The excess Dichromate is titrated with standard Ferrous Ammonium Sulfate, using Orthophenanthroline Ferrous complex as an indicator.

Chi Squared Test - In setting up methods you may want to compare sex or age variables on value for a particular lab test. It is a statistical test most generally suitable for determining whether or not an observed frequency of occurrence differs from that which is expected in accordance with some hypothesis.

Chlorine (Cl) - An element ordinarily existing as a greenish-yellow gas about 2.5 times as heavy as air. At atmospheric pressure and a temperature of - 30.1^{o} F, the gas becomes an amber liquid about 1.5 times as heavy as water.

Chlorine, available - A measure of the total oxidizing power of chlorinated lime and hypochlorites.

Chlorination, breakpoint - Addition of chlorine to water or wastewater until the chlorine demand has been satisfied and further additions result in a residual that is directly proportional to the amount added beyond the breakpoint.

Chlorination, free residual - The application of chlorine or chlorine compounds to water or wastewater to produce a free available chlorine residual directly or through the destruction of ammonia or certain organic nitrogenous compounds.

Chlorine, residual - The amount of chlorine in all forms remaining in water after treatment to ensure disinfection for a period of time.

Coefficient of Variation - An expression of standard deviation in terms as a percentage variance from the mean value. The CV relates the Standard Deviation (SD) to the level at which the measurements are made. CV = SD X 100

Composite - A sample made up of a collection of individual samples obtained at regular intervals.

Composite (proportional) - A composite sample made up of sample whose volume is proportional to the flow at the time of collection.

Confidence Limits - Or confidence interval refers to the upper and lower values of the range (interval) within which random variation are acceptable. Each lab and method may have its own. The Town Branch Lab utilizes ± 2 Standard Deviation, which includes 95% of the values in a Gaussian distribution.

Control - A fluid or substance whose physical and chemical properties closely resembles unknown test specimen whose mean value has been assayed and is used as verification or check on a test procedure.

Chromium (Cr) - Only the trivalent and hexavalent forms of chromium are of biologic significance. Chromium in ambient air originates from industrial sources, are refining, and combustion of fossil fuels.

Copper (Cu) - Wilson's disease is characterized by excessive accumulation of copper in liver, brain, kidneys, and cornea.

Cyanide (CN-) - Cyanide is commonly found in certain rat and pest poisons, metal polishes, photographic solutions and fumigating products.

Density - The ratio of weight (mass) to volume of a material.

DO - Dissolved Oxygen.

EPA – U.S. Environmental Protection Agency.

Iron (Fe) - Acute iron toxicity is nearly always due to accidental ingestion of iron-containing medicines and most often occurs in children.

Filterable Residue - Dissolved solids and colloidal solids.

Fluoridation - The addition of a chemical to increase the concentration of fluoride ions in drinking water to a pre-determined optimum limit to reduce the incidence of dental caries in children.

Frequency distribution - A graphic (usually bar) representation of a set of values relating the number of times each value is obtained to each value. Values are generally listed in ascending order on X-axis and number of times the value occurs on Y-axis.

Gaussian distribution - Normal distribution curve, when data from biologic measurements are plotted according to their frequencies, a bell-shaped curve is usually obtained. In all distributions ± 2 SD from the mean represents 95.45% of the population and ± 3 SD = 99.73%. (1 SD = 68%).

Grab - A single sample of wastewater taken at either a set time or flow.

Gravimetric - A means of measuring unknown concentration of a water quality indicator by weighing.

Halogenated Organics - A general term for organic molecules that contain one or more halogen atoms.

Halogens - Chemical elements, either individually or collectively, composing group VIIB of the periodic table. (i.e. Fluorine, Chlorine, Bromine, Iodine, and Astatine).

Hardness - A characteristic of water, imparted primarily by salts of calcium and magnesium, such as bicarbonates, carbonates, sulfates, chlorides and nitrates, that causes curdling and increased consumption of soap, deposition of scale in boilers, damage in some industrial processes, and sometimes objectionable taste. It may be determined by a standard laboratory titration procedure or computed from the amounts of calcium and magnesium expressed as equivalent calcium carbonate.

Hepatotoxic - Causing liver damage.

LC50 - The concentration of a chemical in air or water that causes death to 50% of the animals.

Lead (Pb) - Lead is the most ubiquitous toxic metal. It is found everywhere, in food, the air, soil and water. Sources include lead-based paint in old dwellings, combustion of lead-containing auto exhausts or industrial emissions. Lead accumulates in bone and teeth. The target organs are the kidneys, nervous and reproductive systems.

Mean - The average or sum of a group of observed values divided by the total number of observations.

Median - The middle value of a series of numbers (arranged in ascending or descending order). Note: If the distribution is not skewed.

Mercury (Hg) - The central nervous system is the major site of toxicity from exposure to elemental mercury.

Milligrams per Liter - A unit of the concentration of water or wastewater constituents. It is 0.001 g of the constituent in 1,000 mL of water. It has replaced the unit formerly used commonly, parts per million, to which it is approximately equivalent, in reporting the results of water and wastewater analysis.

MSDS - Material Safety Data Sheet.

Mutagen - A material that induces genetic changes in the DNA of chromosomes.

Nickel (Ni) - Nickel is a respiratory tract carcinogen.

Nitrogen - An essential nutrient that is often present in wastewater as ammonia, nitrate, nitrite, and organic nitrogen. The concentrations of each form and the sum, total nitrogen, are expressed

as mg/L elemental nitrogen. Also present in some groundwater as nitrate and in some polluted groundwater in other forms.

Nitrogen, ammonia - Quantity of elemental nitrogen present in the form of ammonia (NH_3^-). Ammonia is a chemical combination of Hydrogen (H) and Nitrogen (N) occurring extensively in nature. The combination used in water and wastewater engineering is expressed as NH_3. The protonated form $(NH_4)^+$, coexists with NH_3 water and predominates under conditions of low pH.

Nitrogen Cycle - A graphical presentation of the conservation of matter in nature, form living animal matter through dead organic matter, various stages of decomposition, plant life, and the return of living animal matter, showing changes which occur in course of the cycle. It is used to illustrate biological action and also aerobic and anaerobic acceleration of the transformation of this element by wastewater and sludge treatment.

Nitrogen, Kjeldahl - A standard analytical method used to determine the concentration of the organically-bound ammonia nitrogen state. The method covers the determination of total Kjeldahl nitrogen in surface waters, domestic and industrial wastes, and saline waters. The procedure converts nitrogen components of biological origin such as amino acids proteins and peptides to ammonia, but may not convert the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semi-carbazones and some refractory tertiary amines. Total Kjeldahl is defined as the sum of free ammonia an organic $((NH_4)\ 2S)_4$, under the conditions of digestion.

Nitrogen, Organic - Nitrogen chemically bound in organic molecules such as proteins, amines, and amino acids.

Nitrogen, Organic Kjeldahl - Defined as the difference obtained by subtracting the free ammonia value (of Nitrogen, Ammonia) from the Total Kjeldahl Nitrogen value. This may be determined directly by removal of ammonia before digestion.

Nitrogenous Oxygen - A quantitative measure of the amount of oxygen required for the Demand (NOD) biological oxidation of nitrogenous material, such as ammonia nitrogen and organic carbonaceous oxygen demand has been satisfied.

Non-Filterable Residue - Suspended solids.

Non-Volatile Residue - Fixed solids.

Oil and Grease - In wastewater, a group of substances including fats, waxes, free fatty acids, calcium and magnesium soaps, mineral oils and certain other non-fatty materials. The type of solvent and method used for extraction should be stated for quantization. The method includes the measurement of hexane extractable matter from waters, industrial wastes, and sewages. It is applicable to the determination of relatively non-volatile hydrocarbons, animal fats and waxes, grease and other types of greasy-oily matters. The method is not applicable to measurement of light hydrocarbons that volatilize at temperatures below 80^{o} C. The method covers the range from 5 to 100 mg/L of extractable material.

Organic - Refers to volatile, combustible, and sometimes, biodegradable chemical compounds containing carbon atoms (carbonaceous) bonded together and with other elements. The principal groups of organic substances found in wastewater are proteins, carbon hydrates, and fats and oils.

OSHA - Occupational Safety Health Administration.

ppb - Parts per billion.

ppm - Parts per million.

pH - A measure of the hydrogen-ion concentration in a solution, expressed as the logarithm (base ten) of the reciprocal of the hydrogen-ion concentration in gram moles per liter. On the pH scale (0 - 14), a value of 7 at 25^{o} C represents a neutral condition. Decreasing values, below 7, indicate increasing hydrogen-ion concentration (acidity); increasing values, above 7, indicate decreasing hydrogen-ion concentration (alkalinity). Hydrogen-ion concentration is the weight of hydrogen-ion in moles per liter of solution. Hydrogen-ion concentration is commonly expressed as the pH value, which is the logarithm of the reciprocal of the hydrogen-ion concentration.

Phosphorus - An essential chemical element and nutrient for all life forms. Occurs in orthophosphate, pyrophosphate, tripolyphosphate, and organic phosphate forms. Each of these forms and their sum, total phosphorus, is expressed as mg/L elemental phosphorus. The method covers the determination of specified forms of phosphorus in surface waters, domestic and industrial wastes, and saline waters. They may be applicable to sediment-type samples, sludges, algal blooms, etc., but sufficient data is not available at this time to warrant such usage when measurements for phosphorus content are required. The methods are based on reactions that are specific for the orthophosphate ion. Thus, depending on the prescribed pretreatment of the sample, the various forms of phosphorus shown above may be determined. Except for in-depth and detailed studies, the most commonly measured forms are phosphorus and dissolved phosphorus, and orthophosphate and dissolved orthophosphate. Hydrolysable phosphorus is normally found only in sewage-type samples and insoluble forms of phosphorus, as noted, are determined by calculation. The methods are usable in the 0.01 to 0.5 mg/L P range.

Precision - Ability of self-duplication, amount of spread between replicates.

Range - Measure of dispersion of values and is merely the difference between the largest and the smallest of a group of measurements.

Reagent - Substance used in a chemical reaction to produce another substance or to detect its composition.

Reliability - Measure of a method's ability to achieve both accuracy and precision.

Risk - The probability that a substance will produce harm.

Risk Assessment - Takes into account possible harmful effects on individuals or on society from the use of a material in the quantity and in the manner proposed.

Safety - The reciprocal of risk, probability nothing will happen.

Selenium (Se) - Selenium derivatives are extremely Hepatotoxic.

Shifts - When 6 or more daily values of the same control distribute themselves on one side of the mean value line, but are maintaining a constant level, indicates contamination of standard, use of a new batch of reagents, changes in temperature of water bath, change in spectrophotometer bulb, etc.

Solids - Material in a solid state. In water and wastewater treatment, any dissolved, suspended or volatile substance contained in or removed from water or wastewater.

Solids, Colloidal - Finely divided solids intermediate between dissolved and suspended particles.

Solids, Dissolved - Solids that are present in solution.

Solids, Non-settleable - Wastewater matter that will stay in suspension for an extended period of time. For laboratory purposes, 1 hour.

Solids, Settleable - Matter that will not stay in suspension for 1 hour. Matter that settles to the bottom of an Imhoff Cone within 1 hour.

Solids, Suspended - Matter that is suspended in and will not settle in an hour. For laboratory purposes it is that matter that can be collected on a standard filter.

Solids, Total - The sum of the dissolved and non-dissolved matter in wastewater. The sum of all matter in a wastewater sample.

Solids, Volatile - The quantity of solids in wastewater lost on ignition of dry solids at 550^{o} C. This is a rough approximation of the organic matter.

Solids, Fixed - All matter remaining in a tare after ignition of dry solids at 550^{o} C.

Suspended Solids - Insoluble solids that either float on the surface of, or are in suspension in water, wastewater, or other liquids. Solid organic or inorganic particles (colloidal, dispersed,

coagulated, flocculated) physically held in suspension by agitation or flow. The quantity of material removed from wastewater in a laboratory test, expressed as milligrams per liter and referred to as nonfilterable residue. This method is applicable to surface waters, domestic industrial wastes and saline waters. The practical range of the determination is 20 mg/L to 20,000 mg/L. A well-mixed sample is filtered through a standard glass fiber filter, and the residue retained on the filter is dried to a constant weight at 103 to 105^{o} C. Non-homogenous particulates such as leaves, sticks, fish, and lumps of fecal matter should be excluded from the sample. Preservation of the sample is 4^{o} C for a maximum of 7 days.

Standard - A highly purified material, having a known, weighed, fixed value or concentration of the substance being analyzed.

Standard Deviation - A measure of the scatter of individual values in a Gaussian distribution, the square root of the arithmetic mean of the square of the deviation from the arithmetic mean divided by n-1.

Standard Deviation Interval - Measure of difference between our lab mean and the average of all lab means in a comparison group. The difference is expressed in terms of the SD of all the means in the group.

Tare - A clean, dry container used in a gravimetric analysis.

T-test - Used to ascertain whether the means of two sample groups differ significantly. Calculated using the mean and SD of two matched groups. Refer to Tietz.

TI - Therapeutic Index is the ratio of the dose required to produce a toxic effect.

Total Oxygen Demand (TOD) - a quantitative measure of all oxidizable material in a sample of water or wastewater as determined instrumentally by measuring the depletion of oxygen after high-temperature combustion, as TOC, COD.

Total Carbon (TC) - a quantitative measure (mg/L) of both total inorganic (TIC) and total organic carbon (TOC) in water or wastewater. Determined instrumentally by chemical oxidation to CO_2 and subsequent infrared detection in a carbon analyzer.

Total Organic Carbon - The amount of carbon bound in organic compounds in a sample. Because all organic compounds have carbon as the common element, total organic carbon measurements provide a fundamental means of accessing the degree of organic pollution. The carbonaceous analyzer measures all of the carbon in a sample after injection into the combustion tube. Because of various properties of carbon-containing compounds in liquid samples, preliminary treatment of the sample prior to injection dictates the definition of the carbon as it is measured. The final usefulness of the carbon measurement is in assessing the potential oxygen-demanding load of organic material on a receiving stream. This statement applies whether the carbon measurement is made on a sewage plant effluent, industrial waste, or on water taken directly from the stream. In this light, carbonate and bicarbonate carbon are not a part of the oxygen

demand in the stream and therefore should be discounted in the final calculation or removed prior to analysis. The manner of preliminary treatment therefore defines the types of carbon which are measured.

Toxicant - A substance that kills or injures an organism through chemical or physical or biological action. Examples include cyanides, pesticides, and heavy metals (such as zinc and chromium).

Toxicology - Is the basic science of poisons and their effects. It is the study of the physical effects of chemicals on biological systems.

Trace Element - Any element in water or wastewater that for reasons associated with natural distribution, industrial use, solubility, or other factors, is present at very low concentrations as an essential element.

Trends - When charting quality control values for the same control, the values of the control tended to increase or decrease over a period of 6 consecutive days - can indicate deterioration, changes in stability of standard, incomplete protein precipitation, etc.

Zinc (Zn) - Zinc is ubiquitous in the environment so that it is present in most foodstuffs, water and air.

Section 23
Bibliography

Code of Federal Regulations (CFR). 2003. Guidelines Establishing Test Procedures for the Analysis of Pollutants. 40 CFR 136.3, Title 40, Chapter 1. U.S. Environmental Protection Agency. pg 5-337.

Fisher Scientific. AR50 Fisher Scientific User Manual. Fisher Scientific, Inc.

HACH AutoCat 9000 Chlorine Amperometric Titrator Instruction Manual. HACH Company, Loveland, CO.

HACH DR/4000 Handbook, Method 8083, Procedure Code N.3, Nessler Method following distillation. HACH Company, Loveland, CO.

HACH DR/4000 procedure method 8027. HACH Company, Loveland, CO.

HACH DR/4000 Procedure Manual, EPA approved Method 8190 Phosphorus, Total. HACH Company, Loveland, CO.

HACH HQ Series Portable Meter Users Manual, September 2006, Edition 5. HACH Company, Loveland, CO.

HACH Water Analysis Handbook. Method 8023, Chromium Hexavalent (1,5-Diphenylcarbohydrazide Method). 2nd Edition. 1992. HACH Company, Loveland, CO.

HACH Water Analysis Handbook. Method 8213 Hardness, Total with a Digital Titrator. 2nd Edition. 1992. HACH Company, Loveland, CO.

HACH Water Analysis Handbook. Method 8291, Volatile Acids, Buret Titration Method. 2nd Edition. 1992. HACH Company, Loveland, CO.

Memorandum: EPA Recommendation for the use of HACH method 10360 [Revision 1.1, January 2006] (ATP Case # N04-0013).

Report on the Validation of Proposed EPA Method 360.3 (Luminescence) for the Measurement of Dissolved Oxygen in Water and Wastewater. August 2004. HACH Company, Loveland, CO.

Lachat Micro Dist User Manual, Method Cyanide in Waters (MICRO DIST Cyanide-1). Lachat Instruments, HACH Company, Loveland, CO.

Orion Research Incorporated Laboratory Products Group.
Standard Methods 2320-B. APHA-American Public Health Association Standard Methods for the Examination of Water and Wastewater; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

Standard Methods 2340-C. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

Standard Methods Part 2540-B. Total Solids dried at 103 °C to 105 °C. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

Standard Methods Part 2540-D. Total Suspended Solids Dried at 103 °C to 105 °C. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

Standard Methods Part 2540-E, Fixed and Volatile Solids Ignited at 550 °C. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

Standard Methods Part 2540-F. Settleable Solids. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

Standard Methods Part 3500 B. Chromium, Colorimetric Method. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

Standard Methods 4500-Cl D. Chlorine Residual Amperometric Titration Method. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

Standard Methods Part 4500-P. Phosphorous. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

Standard Methods 5210-A. and 5210-B. Biochemical Oxygen Demand (BOD). APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

Standard Methods Part 5560-C, Distillation Method. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

U.S. EPA. Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

U.S. EPA. Method 130.2. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

U.S. EPA. Method 150.1 pH (Electrometric) Issued 1971 (Editorial revision 1978 and 1982). Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020; U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

U.S. EPA Method 160.2 Residue, Non-Filterable & Total Suspended Solids. Issued 1971. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

U.S. EPA Method 160.3 Residue, Total (Gravimetric, Dried at 103 – 105 ^{o}C). Issued 1971. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

U.S. EPA Method 160.4 Residue, Volatile, (Gravimetric, Ignition at 550 ^{o}C), Issued 1971. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

U.S. EPA. Method 310.1. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

U.S. EPA Method 330.1 Chlorine, Total Residual (Titrimetric, Amperometric) Issued 1978. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

U.S. EPA. Method 335.4. Revision 1.0, August 1993. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

U.S. EPA Method 350.2. Distillation procedure. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

U.S. EPA Method 365.2 Phosphorous, All Forms (Colorimetric, Ascorbic Acid, Single Reagent) Revised March 1983. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

U.S. EPA Method 405.1, Biochemical Oxygen Demand, 5 Days @ 20ºC, Issued 1971, Editorial revision 1974. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S.

Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

U.S. EPA. Microbiological Methods for Monitoring the Environment: Water and Wastes. EPA 600/8-78-017. U.S. Environmental Protection Agency; Environmental Monitoring and Support Laboratory, Office of Research and Development, Washington, DC, 1978. page 124.

Water Pollution Control Federation. Simplified Laboratory Procedures for Wastewater Examination, Third Edition. 1985.

APPENDIX A LABORATORY ANALYSIS LIST

Table A1. Town Branch Laboratory Analysis List

Sample Name	Analysis	Analysis Requirements
Raw Influent	$CBOD_5$	Required
	TSS	Required
	Ammonia	Required
Plant Effluent	pH	Required
	Dissolved Oxygen	Required
	$CBOD_5$	Required
	TSS	Required
	Ammonia	Required
	Total Phosphorus	Required
	Hardness	Required
	Fecal Coliforms	Required
	Residual Chlorine	Required
	Metals - Dissolved	Required
	Metals - Total	Required
Raw Influent	pH	Operation
	Dissolved Oxygen	Operation
	Settleable	Operation
	Total Phosphorus	Operation
	Ortho-phosphate	Operation
	Metals - Dissolved	Operation
	Metals - Total	Operation
Primary Influent	$CBOD_5$	Operation
	Settleable	Operation
	TSS	Operation
Primary Effluent	$CBOD_5$	Operation
	Settleable	Operation
	TSS	Operation
Plant Effluent	Settleable	Operation
	Ortho-phosphate	Operation

Sample Name	Analysis	Analysis Requirements
Creek Above Plant	pH	Operation
	Dissolved Oxygen	Operation
	$CBOD_5$	Operation
	TSS	Operation
	Ammonia	Operation
	Settleable	Operation
	Total Phosphorus	Operation
	Ortho-phosphate	Operation
	Hardness	Operation
	Fecal Coliforms	Operation
	Metals - Total	Operation
Tap Water	Total Phosphorus	Operation
Mixed Liquor	pH	Operation
	Total Alkalinity-Inf.	Operation
	Total Alkalinity-Eff.	Operation
	Settleable	Operation
	MLTSS	Operation
	MLVSS	Operation
	SVI	Operation
	SDI	Operation
	Rise Time	Operation
	Micro Exam	Operation
Return Activated Sludge (R.A.S.)	pH	Operation
	TSS	Operation
	VSS	Operation
	% Total Solids	Operation
Raw Sludge to Thickener	pH	Operation
	% Total Solids	Operation
	% Volatile Solids	Operation
Raw Sludge Thicken Sludge	% Total Solids	Operation
Raw Sludge Thickener Overflow	TSS	Operation
	Total Phosphorus	Operation
Combined Sludge Density Meter	% Total Solids	Operation
	% Volatile Solids	Operation

Sample Name	Analysis	Analysis Requirements
#1 Primary Digester	pH	Operation
	Volatile Acids	Operation
	Total Alkalinity	Operation
	VA/ALK Ratio	Operation
	% Total Solids	Operation
	% Volatile Solids	Operation
#2 Primary Digester	pH	Operation
	Volatile Acids	Operation
	Total Alkalinity	Operation
	VA/ALK Ratio	Operation
	% Total Solids	Operation
	% Volatile Solids	Operation
#3 Primary Digester	pH	Operation
	Volatile Acids	Operation
	Total Alkalinity	Operation
	VA/ALK Ratio	Operation
	% Total Solids	Operation
	% Volatile Solids	Operation
Digested Sludge	pH	Operation
	% Total Solids	Operation
	% Volatile Solids	Operation
	% Removal of Volatile Solids	Operation
Belt Feed and Wastewater		
Belt Feed Solids	% Total Solids	Operation
Filtrate	TSS	Operation
	Total Phosphorus	Operation
Filter Belt Press		
Belt #1 Cake Solids	% Total Solids	Operation
Belt #2 Cake Solids	% Total Solids	Operation
Belt #3 Cake Solids	% Total Solids	Operation
Belt #4 Cake Solids	% Total Solids	Operation
Filter Belt Press Cake	Metals - Total	Operation

Table A2. West Hickman WWTP Analysis List

Sample Name	Analysis	Analysis Requirements
Raw Influent	$CBOD_5$	Required
	TSS	Required
Plant Effluent	$CBOD_5$	Required
	TSS	Required
	Total Phosphorus	Required
	Ammonia	Required
	Hardness	Required
	Fecal Coliforms	Required
	Metals - Dissolved	Required
	Metals - Total	Required
Raw Influent	Ammonia	Operation
	Settleable	Operation
	Total Phosphorus	Operation
	Ortho-phosphate	Operation
	Volatile Acids	Operation
	Total Alkalinity	Operation
	Metals - Dissolved	Operation
	Metals - Total	Operation
Combined Influent	$CBOD_5$	Operation
	TSS	Operation
Biological Phosphorus Removal - Influent	$CBOD_5$	Operation
	Total Phosphorus	Operation
	Ortho-phosphate	Operation
	Volatile Acids	Operation
	Total Alkalinity	Operation
Biological Phosphorus Removal - Effluent	$CBOD_5$	Operation
	Total Phosphorus	Operation
	Ortho-phosphate	Operation
	Volatile Acids	Operation
	Total Alkalinity	Operation

Sample Name	Analysis	Analysis Requirements
Zone #1 Effluent	TSS	Operation
(Formerly Zone #1 M/L)	VSS	Operation
	Total Alkalinity	Operation
		Operation
Zone #2 Effluent	MLTSS	Operation
(Formerly Zone #2 Mixed Liquor)	MLVSS	Operation
	Total Alkalinity	Operation
Plant Effluent	Settleable	Operation
	Ortho-phosphate	Operation
Creek Above Plant	$CBOD_5$	Operation
	TSS	Operation
	Metals - Total	Operation
Creek Below Plant	$CBOD_5$	Operation
	TSS	Operation
	Metals - Total	Operation
Return Activated Sludge	pH	Operation
(R.A.S.)	Total Alkalinity	Operation
	TSS	Operation
	VSS	Operation
	% Total Solids	Operation
	% Volatile Solids	Operation
Belt Feed and Wastewater	% Total Solids	Operation
Combined Sludge Feed	% Volatile Solids	Operation
Filtrate	TSS	Operation
	Total Phosphorus	Operation
	Ortho-phosphate	Operation
<u>Filter Belt Press</u>		
Belt #1 Cake Solids	% Total Solids	Operation
Belt #2 Cake Solids	% Total Solids	Operation
Belt #3 Cake Solids	% Total Solids	Operation
Filter Belt Cake	Metals - Total	Operation

Table A3. Blue Sky WWTP Analysis List

Sample Name	Analysis	Analysis Requirements
Raw Influent	$CBOD_5$	Required
	TSS	Required
	Ammonia	Required
Plant Effluent	$CBOD_5$	Required
	TSS	Required
	Ammonia	Required
	Hardness	Required
	Fecal Coliforms	Required
	Oil & Grease	Required
	Total Phenols	Required
	Total Phosphorus	Required
	Ammonia	Required
	Metals - Dissolved	Required
	Metals - Total	Required
Raw Influent	Total Alkalinity	Operation
	Total Phosphorus	Operation
	Ortho-phosphate	Operation
Plant Effluent	Ortho-phosphate	Operation
Contact Zone	TSS	Operation
	Total Alkalinity	Operation
Re-Aeration	TSS	Operation
	Total Alkalinity	Operation
Digester	TSS	Operation
	Total Alkalinity	Operation

Table A4. Data Provided to the Laboratory by Operations Staff

Town Branch WWTP
- Total Influent Flow
- Peak Flow
- Rainfall

West Hickman WWTP
- Total Influent Flow
- Peak Flow
- Rainfall
- Nitrification Return Flow
- Nitrification Waste Flow
- Total Pounds Chlorine
- Total Pounds SO_2
- Number of Tanks in Service
- Raw Influent - pH
- Raw Influent - DO
- Combined Influent - pH
- Combined Influent - DO
- Zone #1 Effluent - 30 min. Settleable
- Zone #2 Effluent - pH
- Zone #2 Effluent - 30 min. Settleable
- Zone #2 Effluent - Rise Time
- Plant Effluent - pH
- Plant Effluent - DO
- Plant Effluent - TP
- Plant Effluent - Fecal Coliforms
- Chlorine Contact - Residual Chlorine
- Plant Effluent - Residual Chlorine
- Creek Above Plant - pH
- Creek Above Plant - DO
- Creek Below Plant - pH
- Creek Below Plant – DO

Blue Sky WWTP
- Total Influent Flow
- Rainfall
- Raw Influent - pH
- Raw Influent - DO
- Plant Effluent - pH
- Plant Effluent - DO
- Plant Effluent - Residual Chlorine

APPENDIX B STAFF RESPONSIBILITIES

Appendix B. Staff Responsibilities

Class Title: LABORATORY SUPERVISOR

Reports To: Division of Water Quality Director

Supervision Exercised: Laboratory Technicians

General Function:

Manage the operation and maintenance of the government's wastewater laboratory. Performs work of moderate to considerable difficulty in conducting and supervising Laboratory Technicians in a variety of chemical/bacteriological tests and analyses on water released from wastewater treatment plants to determine conformance to EPA mandated standards. Also conducts and supervises Laboratory Technicians in the monitoring, testing and analysis of industrial waste and other discharges into the sanitary sewer systems to determine compliance with local, State and EPA permits and other applicable rules/regulations.

Essential Functions:

Administration/Supervision:

- Set up a laboratory budget and maintain budget records
- Set up and maintain laboratory personnel records - Do employee evaluations. - Deal with employee problems. - Do job interviews for new employees. - Set up employee work schedules.
- Set up and maintain all necessary records and reports as required by the state and EPA.
- Prepares and submits all required records and reports to state and Environmental Protection Agency.
- Do all ordering of supplies and specification of equipment for the laboratory and also for other departments wanting lab related equipment
- Supervises and trains Laboratory Technicians on laboratory and testing procedures
- Supervises the cleaning and sterilization of laboratory equipment, utensils and facilities
- Supervises the storage of all chemicals insuring that all required safety and storage procedures are followed
- Supervises the proper collection of sewage and sludge samples during various phases of wastewater treatment

Program Coordination:

- Manage the LFUCGs laboratories for testing the quality of waters released from three different wastewater treatment plants.
- Maintain and evaluate data on these plants.
- Create and modify programs to sort, store, and calculate data, monitor trends, and generate graphs on personal computer.
- Help other departments set up programs to use data provided by the laboratory. Work with operations staff of three plants and our engineers to help them use the data provided by the laboratory.
- Make suggestions as to how the data could be used to deal with operational problems.

Sample Preparation, Testing and Analysis:

- Check and evaluate all lab data. Run standard to make calibration curve to establish quality of the test.
- Train technicians to run analyses and supervise the procedures.
- Determine how samples are to be prepared for analysis.
- Supervise the sample preparation.
- Determine all laboratory analysis procedures to be used in the analysis of samples. Train the lab personnel on how to use these procedures and supervise their use.
- Set up and run sophisticated bacteriology, chemical, and microbiology analyses that can only be run by the laboratory supervisor; record and report the data.

Miscellaneous:

- Travels to West Hickman WWTP Laboratory to calibrate equipment and/or run analyses on request.
- Maintains special laboratory programs such as safety, quality control or quality assurance.
- Answers telephone inquiries and relays messages and information.
- Responsible for knowing and complying with all UCG and division safety rules and for attending safety meetings. Performs other related duties as required.

Knowledge, Skills and Abilities:

- Considerable knowledge of the principles, practices and techniques of bacteriology and chemistry; the operation and maintenance of complex chemical laboratory equipment; and the safety requirements of a chemical laboratory in the performance of various tests and the storage and use of a variety of chemicals
- Fundamental knowledge of the design and operation of an AA or ICP to facilitate repair and/or assistance in troubleshooting
- Knowledge in the use of micro or personal computers to sort data, monitor trends, generate reports, modify existing and create new programs using spreadsheet applications
- Good knowledge of State and Environmental Protection Agency (EPA) quality control procedures, record keeping and report requirements
- Good observational skills to detect problems in analytical procedures and to detect possible errors in data entry, sample processing, etc.
- Ability to establish and monitor new testing procedures to meet state and EPA requirements
- Ability to use a variety of complex laboratory equipment and understand, set up and run all standard laboratory tests on wastewater, sludge and industrial waste as required by regulatory agencies
- Ability to train and supervise Laboratory Technicians
- Ability to establish and maintain effective working relationships with regulatory agencies, suppliers, industries that discharge waste into the sanitary sewer system and fellow employees.

Communications:

Who (Title)	Communicate About What	How Often
Director	Reports/data	Occasionally
Deputy Director	Reports/date/special requests	Weekly to daily
Municipal Engineer	Reports/data/computers	Daily
Pretreatment Manager	Data/special requests	Daily to weekly
Plant Operations Supervisors	Data/operations	Daily
Sludge Disposal Supervisors	Data/operations	Daily
Engineering Technicians	Special requests	Weekly
Administrative Office Assistants	General/office work	Daily
Vendors/Suppliers	Purchasing / pricing / discount	Weekly
Labs from other cities	Operation Problems	Occasionally
State Inspectors	Operations / paperwork	Monthly
EPA Inspectors	Operations / paperwork	Occasionally
Commercial Labs	Data on an industry	Monthly

Special Requirements:

- May be required to possess a valid driver's license.
- Physical strength and agility to allow considerable walking, lifting, climbing and working with samples that are odorous and hazardous.
- Must be able to work weekends and holidays and perform on-call duty.
- Must be able to operate Urban County Government equipment and vehicles in a safe, prudent and responsible manner.
- All positions require drug testing before employment and will require a pre-employment physical as stated in Ordinances 2114(b) and 23-16.
- Pursuant to the Drug Free Workplace Act of 1988 and to sections 21-52, 22-34 and 23-50 of the Code of Ordinances, all employees must remain drug and alcohol free when reporting to work, while at work and while engaged in any work related activities.
- Based on Federal Regulation 19-10, some positions in this classification are eligible for and will be offered the hepatitis vaccinations. In addition, employees will be required to sign a statement stating they have accepted or declined the hepatitis vaccination.

Class Title: LABORATORY TECHNICIAN

Reports To: Laboratory Supervisor, Plant Operations Supervisor or other supervisor

Supervision Exercised: None

General Function:

Performs technical work of considerable difficulty in conducting a variety of chemical, bacteriological, and industrial waste analyses for wastewater treatment plants to assure compliance with local, State, and Environmental Protection Agency permits.

Essential Functions:

Sample Gathering / Preparation:

- Collects samples and makes sure that samples collected by others are in accordance with government regulations.
- Prepares wastewater, sludge, and pretreatment samples for analyses.
- Cleans laboratory equipment and work area.
- Sterilizes bacteriological equipment.
- Calibrates laboratory equipment.
- Prepares reagents and mixtures as required for laboratory testing.

Sample Testing/Analysis:

- Conducts a variety of laboratory analyses in accordance with government regulations to include pH, biochemical, oxygen demand, suspended and total solids, volatile solids, chemical concentrations, alkalinity and other.
- Conducts routine bacteriological examination on wastewater samples. Conducts microorganism counts of activated sludge.
- Makes dissolved oxygen and pH readings in the field when requested.

Recording/Reporting Results:

- Records all data on worksheets and monthly sheets.
- Prepares monthly reports.
- Maintains inventory of supplies by informing Laboratory Supervisor when inventories get low.

Miscellaneous:

- Travels to West Hickman WWTP Laboratory to calibrate equipment and/or run analyses on request.
- Maintains special laboratory programs such as safety, quality control or quality assurance.
- Answers telephone inquiries and relays messages and information.
- May be assigned to perform duties of higher or lower level classifications in this or related class series and/or perform duties on a temporary or acting basis in accordance

with Ordinances 21-15 and 21-16 and/or act as a lead worker.
- Responsible for knowing and complying with all UCG and division safety rules and for attending safety meetings. Performs other related duties as required.

Physical Demands and Working Conditions:

- Physical demands include standing, lifting, carrying, and walking.
- Must have the strength and agility to allow the employee to perform job duties which would be found at the level of medium work (i.e. exerting 20 to 50 pounds of force) as defined in the PAQ.
- Working conditions include performing a majority of job duties indoors with wet and humid surroundings, fumes, odors, and chemicals.

Knowledge, Skills and Abilities:

Considerable knowledge of:
- the occupational hazards and safety precautions necessary relative to the area of assignment;
- the elementary principles, practices and techniques of bacteriology and chemistry;
- the operation, maintenance and calibration of standard and complex chemical laboratory equipment.

Good knowledge of:
- the elementary principles, practices and techniques of bacteriology and chemistry;
- personal computers and related software used in the field;
- the operation and care of standard chemical laboratory equipment.

Ability to:
- perform standard chemical and bacteriological tests accurately;
- follow oral and written instructions exactly;
- prepare reports on the results of laboratory tests performed;
- establish and maintain effective working relationships with fellow employees and other involved parties;
- understand, set up and run standard laboratory tests on wastewater and sludge per "standard methods".

Special Requirements:

- May be required to possess a valid driver's license.
- Physical strength and agility to allow considerable walking, lifting, climbing and working with samples that are odorous and hazardous.
- Must be able to work weekends and holidays and perform on-call duty.
- Must be able to operate Urban County Government equipment and vehicles in a safe, prudent and responsible manner.
- All positions require drug testing before employment and will require a pre-

employment physical as stated in Ordinances 2114(b) and 23-16.

- Pursuant to the Drug Free Workplace Act of 1988 and to sections 21-52, 22-34 and 23-50 of the Code of Ordinances, all employees must remain drug and alcohol free when reporting to work, while at work and while engaged in any work related activities.
- Based on Federal Regulation 19-10, some positions in this classification are eligible for and will be offered the hepatitis vaccinations. In addition, employees will be required to sign a statement stating they have accepted or declined the hepatitis vaccination.

APPENDIX C CURRENT PERSONNEL AND LAB APPROVED SIGNATURES

Appendix C. Current Personnel and Laboratory's Approved Signatures

Job Title: Laboratory Supervisor

Name: Dr. David J. Price

Employee #: 46274

Education: Ph.D. Biology, University of Kentucky, 2008

Signature: _____

Job Title: Laboratory Technician - QA/QC Manager

Name: La Vada M. Green

Employee #: 22849

Education: B.S. Physics, Eastern Kentucky University,

Signature: _____

Job Title: Laboratory Technician – Safety Officer

Name: Di-Linh Cao-Nguyen

Employee #: 39494

Education: B.S. Biology, University of Kentucky, 1997

Signature: _____

Job Title: Laboratory Technician - Microbiology

Name: Maria Lundin

Employee #: 45854

Education: B.S. Biology, University of Wisconsin-Green Bay, 1995

Signature: _____

Job Title: Laboratory Technician

Name: Jerry W. McDaniel

Employee #: 35894

Education: Navy training, Medical Technology, 1974

Signature: _____

Job Title: Laboratory Technician – Database management

Name: Brian Reynolds

Employee #: 43385

Education: B.S. Biology, Eastern Kentucky University, 2006

Signature: _____

TOWN BRANCH LABORATORY STANDARD OPERATING PROCEDURES

Alkalinity (Titrimetric)
Standard Methods 2320-B

1. Scope, Significance to Process and Application

1.1 This method is applicable to drinking, surface, and saline waters, as well as, domestic and industrial wastes.

2. Summary of Method

2.1 Executive Summary

The principle of operation for the Orion Test Kit is the same as the conventional titration. A pre-measured volume of reagent is added to the sample. This reagent is composed of several acids that react with the alkaline species in the sample, resulting in a change in sample pH. The observed pH reading after the addition of the reagent varies directly with the total alkalinity. Each pH reading corresponds to a unique value for alkalinity, expressed in mg/L (ppm) $CaCO_3$. The alkalinity values are obtained in a chart that cross references with the pH values.

2.2 Discussion

Alkalinity of water is its acid-neutralizing capacity. Raw domestic wastewater has an alkalinity less than or slightly higher than in the water supply. Properly operating anaerobic digesters have a supernatant alkalinity in the range of 2000-4000 mg $CaCO_3$/L. Some samples are diluted. No color change is noted.

3. Health & Safety Precautions

3.1 Watch out for broken glass from beakers and cylinders.
3.2 Wastewater samples have the potential to be hazardous, use appropriate caution.

4. Sample Handling and Preservation

4.1 Samples should be run as soon as possible.

5. Reagents

5.1 Total Alkalinity Reagent (Orion # 700011)
5.2 Alkalinity Standard/Control (1000 ppm; Orion # 700012)
5.3 Nanopure Water

6. Equipment & Lab Ware

6.1 Fisher AR50 ph Meter using a glass electrode and reads to 0.05 pH units
6.2 100 mL volumetric flask (for standard)
6.3 100 mL graduated cylinders
6.4 Appropriate size beakers to contain samples and reagents
6.5 Repipettors (1 mL, 5 mL, and 10 mL) and tips

7. Interferences

7.1 Substances, such as salts of weak organic and inorganic acids present in large amounts, may cause interference in the electrometric pH measurements.
7.2 Oil and Grease, by coating the pH electrode may also interfere, causing a sluggish response.

8. Procedures

8.1 Steps

1) Calibrate pH meter (see SOP-pH, Section 8).

2) Check alkalinity of Nanopure water. (Any alkalinity in the water used for diluting will contribute to the total alkalinity measured in the control)

   a. Measure 100 mL of Nanopure water into 150 mL beaker.
   b. Add 1.0 mL Alkalinity Reagent and insert pH probe.
   c. Turn on stirrer.
   d. Read pH value when the meter displays “STABLE”.
   e. Determine total alkalinity of the Nanopure water using the Low-level chart (0-25 ppm), and multiply result by 0.9. This value (AH_2O) is the contribution to total alkalinity from Nanopure water.

3) Check alkalinity of Standard.

   a. Measure 10 mL of Alkalinity Standard into 100 mL volumetric flask and dilute to mark with Nanopure water.
   b. Pour standard into 150 mL beaker.
   c. Add 10 mL of Total Alkalinity Reagent and insert pH probe.
   d. Turn on stirrer.
   e. Read pH value when the meter displays “STABLE”.
   f. Obtain alkalinity value (A) from the Full chart (0-225 ppm) by cross referencing with pH value.
   g. Determine alkalinity of standard (A_{std}):

$$A_{std} = A - AH_2O \quad \text{(Value should be } 100 \pm 5 \text{ ppm).}$$

4) Record alkalinity value, date, time and initials in bench sheet.

5) For samples:

a. Determine sample volume from the bench sheet (generally, 100 mL).
b. To make a 50:1 dilution: pipette 2 mL of sample and dilute to 100 mL volume with Nanopure water.
c. Pour sample into 150 mL beaker.
d. Add 10 mL of Total Alkalinity Reagent and insert pH probe.
e. Turn on stirrer.
f. Read pH value when the meter displays "STABLE".
g. Obtain alkalinity value (A_{smp}) from the Full chart (0-225 ppm) by cross referencing with pH value.
h. If diluted, determine alkalinity of diluted sample (A_{ds}):

$$A_{ds} = A_{smp} - AH_2O$$

6) Record alkalinity value, date, time and initials in bench sheet.

8.2 Helpful Hints

1) Alkalinity ranges for the standard should be 100 ± 5 ppm, if not, try recalibrating and use clean glassware.

9. QA/QC Requirements

Analysis values for the standard must have a pH range of 4.41 ± 0.05 (equivalent to 930 - 1070 mg/L $CaCO_3$). If this criterion is not met, corrective action is indicated. See Quality Assurance Program (QAP) Sec. 15 "Corrective Action Policies and Procedures".

10. Expected Results

10.1 KPDES Permit Requirements

None required.

10.2 Process Ranges (Mean ± SD)

1) Town Branch WWTP

Mixed Liquor Influent	218 ± 11.0
Mixed Liquor Effluent	152 ± 16.8
Digesters	3270.2 ± 245.8

2) West Hickman WWTP

Raw Effluent	214 ± 27.1
Zone #1 Effluent	177 ± 10.7
Zone #2 Effluent	170 ± 15.8
Return Activated Sludge	284 ± 26.2

11. Data Analysis and Calculations

11.1 Calculations determined by dilution.
11.2 See following pages for alkalinity concentration tables.

12. Bibliography

12.1 U.S. EPA. Method 310.1. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

12.2 Standard Methods 2320-B. APHA-American Public Health Association Standard Methods for the Examination of Water and Wastewater; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

12.3 Total Alkalinity Measurement in Natural Waters. Application Information Procedure 517. Thermo Fisher Scientific Inc. http://www.thermo.com/com/cda/products/product_application_details/1,,11636,00.html

ORION Total Alkalinity Test Kit
LOW RANGE 0 to 25 mg/L as CaCO3

Add 1 mL of ORION Total Alkalinity Reagent to 100 mL and mix well. Measure pH of mixture and read total alkalinity from this table.

Observed pH	Total Alkalinity	Observed pH	Total Alkalinity	Observed pH	Total Alkalinity	Observed pH	Total Alkalinity	Observed pH	Total Alkalinity
4.00	0.0	4.35	5.4	4.70	10.9	5.05	16.4	5.40	21.9
4.01	0.1	4.36	5.6	4.71	11.1	5.06	16.6	5.41	22.1
4.02	0.3	4.37	5.8	4.72	11.3	5.07	16.7	5.42	22.2
4.03	0.4	4.38	5.9	4.73	11.4	5.08	16.9	5.43	22.4
4.04	0.6	4.39	6.1	4.74	11.6	5.09	17.1	5.44	22.6
4.05	0.7	4.40	6.2	4.75	11.7	5.10	17.2	5.45	22.7
4.06	0.9	4.41	6.4	4.76	11.9	5.11	17.4	5.46	22.9
4.07	1.1	4.42	6.5	4.77	12.0	5.12	17.5	5.47	23.0
4.08	1.2	4.43	6.7	4.78	12.2	5.13	17.7	5.48	23.2
4.09	1.4	4.44	6.9	4.79	12.4	5.14	17.8	5.49	23.3
4.10	1.5	4.45	7.0	4.80	12.5	5.15	18.0	5.50	23.5
4.11	1.7	4.46	7.2	4.81	12.7	5.16	18.2	5.51	23.7
4.12	1.8	4.47	7.3	4.82	12.8	5.17	18.3	5.52	23.8
4.13	2.0	4.48	7.5	4.83	13.0	5.18	18.5	5.53	24.0
4.14	2.1	4.49	7.6	4.84	13.1	5.19	18.6	5.54	24.1
4.15	2.3	4.50	7.8	4.85	13.3	5.20	18.8	5.55	24.3
4.16	2.5	4.51	8.0	4.86	13.4	5.21	18.9	5.56	24.4
4.17	2.6	4.52	8.1	4.87	13.6	5.22	19.1	5.57	24.6
4.18	2.8	4.53	8.3	4.88	13.8	5.23	19.3	5.58	24.7
4.19	2.9	4.54	8.4	4.89	13.9	5.24	19.4	5.59	24.9
4.20	3.1	4.55	8.6	4.90	14.1	5.25	19.6	5.60	25.1
4.21	3.2	4.56	8.7	4.91	14.2	5.26	19.7		
4.22	3.4	4.57	8.9	4.92	14.4	5.27	19.9		
4.23	3.6	4.58	9.1	4.93	14.5	5.28	20.0		
4.24	3.7	4.59	9.2	4.94	14.7	5.29	20.2		
4.25	3.9	4.60	9.4	4.95	14.9	5.30	20.4		
4.26	4.0	4.61	9.5	4.96	15.0	5.31	20.5		
4.27	4.2	4.62	9.7	4.97	15.2	5.32	20.7		
4.28	4.3	4.63	9.8	4.98	15.3	5.33	20.8		
4.29	4.5	4.64	10.0	4.99	15.5	5.34	21.0		
4.30	4.7	4.65	10.2	5.00	15.6	5.35	21.1		
4.31	4.8	4.66	10.3	5.01	15.8	5.36	21.3		
4.32	5.0	4.67	10.5	5.02	16.0	5.37	21.5		
4.33	5.1	4.68	10.6	5.03	16.1	5.38	21.6		
4.34	5.3	4.69	10.8	5.04	16.3	5.39	21.8		

ORION Total Alkalinity Test Kit
HIGH RANGE 0 to 225 mg/L as CaCO3

Add 10 mL of ORION Total Alkalinity Reagent to 100 mL and mix well. Measure pH of mixture and read total alkalinity from this table.

Observed pH	Total Alkalinity	Observed pH	Total Alkalinity	Observed pH	Total Alkalinity	Observed pH	Total Alkalinity	Observed pH	Total Alkalinity
3.66	0.0	4.01	47	4.36	93	4.71	140	5.06	187
3.67	1.2	4.02	48	4.37	95	4.72	141	5.07	188
3.68	2.5	4.03	49	4.38	96	4.73	143	5.08	190
3.69	3.8	4.04	51	4.39	97	4.74	144	5.09	191
3.70	5.2	4.05	52	4.40	99	4.75	145	5.10	192
3.71	6.5	4.06	53	4.41	100	4.76	147	5.11	194
3.72	7.8	4.07	55	4.42	101	4.77	148	5.12	195
3.73	9.2	4.08	56	4.43	103	4.78	149	5.13	196
3.74	11	4.09	57	4.44	104	4.79	151	5.14	198
3.75	12	4.10	59	4.45	105	4.80	152	5.15	199
3.76	13	4.11	60	4.46	107	4.81	153	5.16	200
3.77	15	4.12	61	4.47	108	4.82	155	5.17	202
3.78	16	4.13	63	4.48	109	4.83	156	5.18	203
3.79	17	4.14	64	4.49	111	4.84	157	5.19	204
3.80	19	4.15	65	4.50	112	4.85	159	5.20	206
3.81	20	4.16	67	4.51	113	4.86	160	5.21	207
3.82	21	4.17	68	4.52	115	4.87	161	5.22	208
3.83	23	4.18	69	4.53	116	4.88	163	5.23	210
3.84	24	4.19	71	4.54	117	4.89	164	5.24	211
3.85	25	4.20	72	4.55	119	4.90	165	5.25	212
3.86	27	4.21	73	4.56	120	4.91	167	5.26	214
3.87	28	4.22	75	4.57	121	4.92	168	5.27	215
3.88	29	4.23	76	4.58	123	4.93	169	5.28	216
3.89	31	4.24	77	4.59	124	4.94	171	5.29	218
3.90	32	4.25	79	4.60	125	4.95	172	5.30	219
3.91	33	4.26	80	4.61	127	4.96	173	5.31	220
3.92	35	4.27	81	4.62	128	4.97	175	5.32	222
3.93	36	4.28	83	4.63	129	4.98	176	5.33	223
3.94	37	4.29	84	4.64	131	4.99	177	5.34	224
3.95	39	4.30	85	4.65	132	5.00	179	5.35	226
3.96	40	4.31	87	4.66	133	5.01	180		
3.97	41	4.32	88	4.67	135	5.02	181		
3.98	43	4.33	89	4.68	136	5.03	183		
3.99	44	4.34	91	4.69	137	5.04	184		
4.00	45	4.35	92	4.70	139	5.05	186		

Nitrogen, Ammonia
HACH Method Salicylate Method 10205
TNT+ 830, ULR (0.015 to 2.000 mg/L NH_3–N)
TNT+ 831, LR (1 to 12 mg/L NH_3–N)
TNT+ 832, HR (2 to 47 mg/L NH_3–N)
EPA Method 350.1

1. Scope, Significance to Process and Application

1.1 Ammonia concentrations in wastewater samples are an indication of nutrient levels in the wastewater process stream. The reduction of ammonia levels throughout the wastewater treatment process is highly important as plant effluent nutrient concentrations must be low enough (See Sec. 10.1 Permit Requirements) so as to avoid detrimental effect on the receiving environment. Low-level ammonia nitrogen may be present in water naturally as a result of the biological decay of plant and animal matter. Higher concentrations may be found in raw sewage and industrial effluents. High concentrations in surface waters can indicate contamination from waste treatment facilities, industrial effluents or fertilizer runoff. Excessive ammonia concentrations are toxic to aquatic life, and can exert an undesirable oxygen demand on the receiving stream.

2. Summary of Method

2.1 Executive Summary

Ammonium ions react at pH 12.6 with hypochlorite ions and salicylate ions in the presence of sodium nitroprusside as a catalyst to form indophenol. The amount of color formed is directly proportional to the ammonia nitrogen present in the sample. Test results are measured at 690 nm. Nitrogen Ammonia analysis at Town Branch Laboratory refers to the spectrophotometric analysis of nitrogen ammonia compounds in a water/wastewater sample.

2.2 Discussion

This method covers the determination of ammonia-nitrogen exclusive of total nitrogen, in drinking, surface and saline waters, domestic and industrial wastes. ULR HACH method covers the range from 0.015 to 2.000 mg/L NH_3-N. The samples are analyzed colorimetrically with a HACH DR/5000.

3. Health & Safety Precautions

3.1 Watch out for broken glass from beakers and cylinders.
3.2 Wastewater samples have the potential to be hazardous, use appropriate caution.

4. Sample Handling and Preservation

4.1. Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis.
4.2. Preserve the samples by reducing the pH to 2 or less with at least 2 mL of Hydrochloric Acid.
4.3. Store at 4 °C (39 °F) or less.
4.4. Preserved samples may be stored up to 28 days.
4.5. Before analysis, warm stored samples to 20–23 °C (68–73.4 °F) and neutralize to pH 7.0 with 5.0 N Sodium Hydroxide.
4.6. Correct the test result for volume additions.

5. Reagents and Lab Equipment

5.1. Ammonia, TNTplus ULR Reagent Set (HACH TNT830)
5.2. Ammonia, TNTplus LR Reagent Set (HACH TNT831)
5.3. Ammonia, TNTplus HR Reagent Set (HACH TNT832)
5.4. Pipetors (100-1000 µL, 1–5 mL)
5.5. Pipet Tips
5.6. Nitrogen Ammonia Standard Solution, 1.0-mg/L NH_3–N (HACH 189149)
5.7. Nanopure Water
5.8. Hydrochloric Acid Standard Solution, 1N
5.9. Hydrochloric Acid, concentrated ACS
5.10. Sodium Hydroxide Standard Solution, 1N (HACH 104532)
5.11. Test Tube Rack for 13-mm vial
5.12. DRB200 Reactor, 115 V, 9x13mm

6. Interferences

6.1. The ions listed in the Interfering substances table have been individually tested up to the given concentrations and do not cause interference. The cumulative effects of these ions or the influence of other ions have not been determined.
6.2. Primary amines are determined and cause high-bias results. A 10,000-fold excess of urea does not interfere. All reducing agents interfere and cause low-bias results.
6.3. **Important Note:** An analyte concentration greatly in excess of the stated range will adversely affect color formation, resulting in a false reading within the method range.
6.4. Measurement results can be verified using sample dilutions or standard additions.
6.5. Samples with severe interferences require distillation. Perform the distillation procedure using the HACH General Purpose Distillation Set.

7. Procedures

7.1 TNT 830 ULR (0.015 to 2.000 mg/L NH_3–N)

1) Carefully remove the protective foil lid from the DosiCap™ Zip. Unscrew the cap from the vial.
2) Carefully pipet 5.0 mL of sample into the vial. Immediately proceed to step 3.
3) Flip the DosiCap Zip over so that the reagent side faces the vial. Screw the cap tightly onto the vial.
4) Shake the capped vial 2–3 times to dissolve the reagent in the cap. Verify that the reagent has dissolved by looking down through the open end of the DosiCap Zip.
5) Wait 15 minutes.
6) After 15 minutes, invert the sample an additional 2–3 times to mix. The color remains constant for an additional 15 minutes after the timer expires.
7) Thoroughly clean the outside of the vial with a Kim-wipe.
8) Insert the prepared vial into the DR5000 cell holder. Slide the lid closed. The instrument reads the barcode, then selects and performs the correct test. Results are in mg/L NH_3–N.

7.2 TNT 831 LR (1 to 12 mg/L NH_3–N)

1) Carefully remove the protective foil lid from the DosiCap™ Zip. Unscrew the cap from the vial.
2) Carefully pipet 0.5 mL (500 µL) of sample into the vial. Immediately proceed to step 3.
3) Flip the DosiCap Zip over so that the reagent side faces the vial. Screw the cap tightly onto the vial.
4) Shake the capped vial 2–3 times to dissolve the reagent in the cap. Verify that the reagent has dissolved by looking down through the open end of the DosiCap Zip.
5) Wait 15 minutes.
6) After 15 minutes, invert the sample an additional 2–3 times to mix.
7) The color remains constant for an additional 15 minutes after the timer expires.
8) Thoroughly clean the outside of the vial with a Kim-wipe .
9) Insert the prepared vial into the DR5000 cell holder. Slide the lid closed. The instrument reads the barcode, then selects and performs the correct test. Results are in mg/L NH_3–N.

7.3 **TNT 832 HR (2 to 47 mg/L NH_3–N)**

1) Carefully remove the protective foil lid from the DosiCap™ Zip. Unscrew the cap from the vial.
2) Carefully pipet 0.2 mL (200 µL) of sample into the vial. Immediately proceed to step 3.
3) Flip the DosiCap Zip over so that the reagent side faces the vial. Screw the cap tightly onto the vial.
4) Shake the capped vial 2–3 times to dissolve the reagent in the cap. Verify that the reagent has dissolved by looking down through the open end of the DosiCap Zip.
5) Wait 15 minutes.
6) After 15 minutes, invert the sample an additional 2–3 times to mix.
7) The color remains constant for an additional 15 minutes after the timer expires.
8) Thoroughly clean the outside of the vial with a Kim-wipe.
9) Insert the prepared vial into the DR5000 cell holder. Slide the lid closed. The instrument reads the barcode, then selects and performs the correct test. Results are in mg/L NH_3–N.

7.4 **Reagent blanks**

A reagent blank can be measured and the value subtracted from the results of each test performed in same reagent lot. Use deionized water in place of sample in the Salicylate method, TNTplus 830, 831, or 832 test.

To subtract the value of the blank from a series of measurements:

1. Measure the blank per step 3.
2. Turn on the reagent blank option.
3. The measured value of the blank should be displayed in the highlighted box. Accept this value.

The reagent blank value will now be subtracted from all results until the function is turned off or a different method is selected. Alternately, the blank can be recorded and entered at any later time by pressing the highlighted box and using the keypad to enter the value.

7.5 **Sample blanks**

Colored or turbid samples can cause high results. To compensate for color or turbidity the procedure is repeated without the addition of the color forming reagent that is present in the DosiCap Zip.

To determine the sample blank:

1. Run the Salicylate method, TNTplus 830, 831, or 832 test, but do not remove the foil from the DosiCap Zip in step 1.
2. Replace the cap in its original position in step 3.
3. Subtract the value obtained in step 8 from the value obtained on the original sample to give the corrected sample concentration.

Samples without color or turbidity do not require sample blanks.

7.6 Helpful Hints

1) After washing used glassware, all glassware must be thoroughly rinsed with lab grade water.

2) Analysis results are directly proportional to sample volumes therefore it is very important that sample volume measurements are accurate.

3) Ammonia TNTplus tubes are temperature sensitive and must be stored in the refrigerator (4^o C) when not in use.

8. Standard Preparation

8.1. ULR Low Standard (1.01 mg/L NH_3–N)
Combine 1.00 mL of 10.1 mg/L standard + 9.0 mL DI water

8.2. HR High Standard (10.1 mg/L NH_3–N)
Add 20.0 mL of stock ammonia standard solution (100 ppm NH_3–N) to 200 mL volumetric flask. Dilute to 200 mL with Nanopure water.

9. QA/QC Requirements

9.1 A low standard (1.01 mg/L) and a high standard (10.1 mg/L) must be analyzed with every analytical run.

9.2 5% of all samples must be run in duplicate. Duplicate concentration values should agree within 5%.

9.3 Data acceptance criteria: Analysis values for Standards must agree within 10% of the standard's known value and duplicate values must agree within 5%. If these criteria are not met, corrective action is indicated. See Quality Assurance Program (QAP) Sec. 15 "Corrective Action Policies and Procedures".

10. Expected Results

10.1 KPDES Permit Requirements

1) Town Branch Wastewater Treatment Plant effluent limitations:

Weekly average = 3 mg/L (May 1 - October 31)
Monthly average = 2 mg/L (May 1 - October 31)

Weekly average = 10.5 mg/L (November 1 - April 30)
Monthly average = 7 mg/L (November 1 - April 30)

2) West Hickman Wastewater Treatment Plant effluent limitations:

Weekly average = 6 mg/L (May 1 - October 31)
Monthly average = 4 mg/L (May 1 - October 31)

Weekly average = 15 mg/L (November 1 - April 30)
Monthly average = 10 mg/L (November 1 - April 30)

In the event that analysis results indicate values greater then KPDES permit requirements, retest. If the value indicated by the retest is greater than KPDES permit requirements, **Immediately** notify the Plant Superintendent and the Laboratory Supervisor.

10.2 Process Ranges

Typical ammonia values vary a great deal throughout the wastewater treatment process, depending on sample location and environmental conditions (i.e. high rainfall events).

The following are typical Ammonia values found in process waters:

Raw Influent	0 mg/L to ≈ 25 mg/L
Plant Effluent	0 mg/L to 2.0 mg/L

11. Data Analysis and Calculations

11.1 Concentrations are read directly from the DR5000 spectrophotometer. Ensure that test results are corrected for volume dilutions.

12. Bibliography

12.1 U.S. EPA Method 350.1. Nitrogen, Ammonia, Colorimetric, Automated Phenate. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

12.2 HACH. DOC316.53.01081, Method 10205 Nitrogen-Ammonia Salicylate method, TNTplus 830. HACH Company, Loveland, CO, 2008.

12.3 HACH. DOC316.53.01082, Method 10205 Nitrogen-Ammonia Salicylate method, TNTplus 831. HACH Company, Loveland, CO, 2008.

12.4 HACH. DOC316.53.01083, Method 10205 Nitrogen-Ammonia Salicylate method, TNTplus 832. HACH Company, Loveland, CO, 2008.

Biochemical Oxygen Demand (BOD_5) and Carbonaceous Biochemical Oxygen Demand ($CBOD_5$) Analysis
EPA Method 405.1 (Editorial Revision 1974)
Standard Methods 5210-A and 5210-B

1. Scope, Significance to Process and Application

The Biochemical Oxygen Demand (BOD_5) and Carbonaceous Biochemical Oxygen Demand ($CBOD_5$) analysis assesses the concentration and general composition of organic matter in raw water supplies, wastewaters, treated effluents, and receiving waters. This test is used to determine the efficiency of the treatment process at Town Branch, West Hickman, and Blue Sky Wastewater Treatment Plants.

2. Summary of Method

2.1 Executive Summary

An initial dissolved oxygen (DO) reading is taken of the sample (or dilution). The sample is then incubated in the dark at 20ºC for 5 days. A final DO reading is then taken. Initial and final dissolved oxygen values are entered into a spreadsheet that calculates the BOD and CBOD values which are expressed in milligrams per liter.

2.2 Discussion

The BOD_5 test measures the amount of oxygen uptake caused by both the biodegradation by micro-organisms of organic materials in wastewaters (carbonaceous demand) and the oxidation of nitrogen forms in wastewaters (nitrogenous demand) over a 5 day incubation period, coupled with calculations to derive the Biochemical Oxygen Demand (BOD) of the sample tested. The $CBOD_5$ test involves the addition of a nitrification inhibitor to exclude nitrogenous demand allowing the measurement of just the carbonaceous demand (CBOD).

3. Health & Safety Precautions

3.1 All municipal and industrial wastewaters are potentially hazardous. Gloves and goggles should be worn when dispensing these samples.

4. Sample Handling and Preservation

4.1 Collect sample in plastic or glass and store at 4ºC.
4.2 Run analysis within 48 hours.
4.3 Grab samples:
If tested within 2 hours of collection, no cooling is needed.
If testing cannot start within 2 hours, cool to 4ºC and test within 6 hours.

4.4 Composite samples:
Use the same criteria as for grab samples starting at the end of compositing. Samples should be at 4ºC. At no time should an analyst test a 24 hour composite sample after the 48 hour holding period.

5. Reagents

5.1 Sodium Sulfite solution, 0.025 N
Instill 0.1575 g ± 0.0001 g Sodium sulfite into 100 mL volumetric flask and bring to mark with Nanopure lab water (Note: This solution is not stable - prepare daily)
5.2 HACH BOD Nutrient Buffer Pillows, 3 L and 6 L
5.3 BOD Seed Inoculum (Polyseed®)
5.4 NCL Glucose-Glutamic Acid BOD Standard (198 ± 30.5 mg/L)
5.5 HACH Nitrification Inhibitor with dispenser cap
5.6 Nanopure laboratory water

6. Equipment & Lab Ware

6.1. 300 mL disposable BOD bottles (Environmental Express)
6.2. BOD bottle stoppers and plastic caps
6.3. HACH HQ40d Portable Meter with LBOD101 probe
6.4. Control Co® Digital Barometer
6.5. Fisher Scientific® Isotemp Incubator *Model 304* at 20ºC ± 1ºC
6.6. 9 L glass dilution water bottle
6.7. Siphon hose (silver treated blue hose) and valve
6.8. Graduated cylinders to measure samples (250 and 500 mL)
6.9. Squeeze bottle for dispensing dilution water
6.10. Squeeze bottle with lab water to rinse probe between initial DO readings
6.11. 500 mL and 1 L Erlenmeyer flasks
6.12. 10 mL and 500 mL beakers
6.13. Barant Co® vacuum/pressure aeration system with hose and diffuser
6.14. Labsystems® adjustable 1-5 mL finnpipette

7. Interferences

7.1 Greases and oils in the sample.
7.2 Non representative particles such as leaves, sticks and debris.
7.3 Temperature differentials (*i.e*. ambient temperature DO probe used to analyze a colder sample).
7.4 Air entrapped in the BOD bottle (bubbles).

8. Procedures

8.1 Steps

8.1.1 Preparation of BOD stoppers

1) BOD stoppers are washed with detergent and tap water.
2) Stoppers are then soaked in HCl (1:1) for at least 1 h.
3) Stoppers are then soaked in Nanopure water.
4) Allow to air-dry.
5) Wrap stoppers in blue sterile paper and indicator tape.
6) Autoclave wrapped stoppers.
7) Stoppers are maintained in sterile wrap until ready to use.

8.1.2 Preparation of BOD bottles

1) Obtain correct number of Env. Express disposable 300 mL BOD bottles (Use only once)**.**
2) Using a permanent marker, label bottles in accordance with bench sheet.
3) To each sample BOD bottle add 2 shots (cycle the dispenser twice) of HACH® Nitrification Inhibitor. *Do not add Nitrification Inhibitor to the unseeded blank, the blanks, or to industrial samples.*

8.1.3 Preparation of dilution water

1) Place 9 L of Nanopure water into a 10 L dilution bottle.
2) Place filled dilution bottle on stir plate inside incubator (20 ± 1° C).
3) Add a 3 L pillow and a 6 L pillow of BOD nutrient buffer into the dilution water bottle (1 mL nutrient to 1 L of lab water)
4) Mix thoroughly using a magnetic stirrer.

8.1.4 Preparation of BOD seed

1) Siphon 500 mL dilution water into 500 mL graduated cylinder.
2) Pour the 500 mL of dilution water into 1 L Erlenmeyer flask.
3) Pull apart Polyseed® capsule and pour contents into flask.
4) Submerge the aerator discharge diffuser in the flask using a support stand to hold the diffuser centered at the bottom of the flask, turn the aerator on.
5) Aerate contents of flask for at least one hour.
6) Decant the supernatant carefully into a clean 500 mL beaker so as not to allow any bran in the remaining seed solution.
7) Stir using a spin bar and magnetic stir table.
8) The Polyseed® solution should be used within 6 hours of rehydration of the capsule's contents.

8.1.5 Siphon dilution water into a labeled squirt bottle for dispensing.

8.1.6 Preparation of HACH HQ40d Meter with LBOD101 probe

1) Calibrate DO meter at the beginning of the analysis day (see SOP-DO, Section 8).
2) Meter must be calibrated a minimum of once per analysis day. Recalibrate meter if reading are taken in the afternoon.
3) Rinse LBOD probe between samples with Nanopure water.

8.1.7 Blank Control Samples

1) Prepare four BOD bottles as follows:
   label one bottle "Unseeded Blank" - NO seed will be added
   label one bottle "Blank 1" and add 2 mL of seed solution
   label one bottle "Blank 2" and add 3 mL of seed solution
   label one bottle "Blank 3" and add 4 mL of seed solution
2) Siphon dilution water into BOD bottles until full.
3) Measure initial DO for each and record in bench sheet.
4) Replace any displaced dilution water, stopper, cap, and incubate at 20 ± 1^o C for 5 days.
5) The 5-day BOD at 20ºC should have a depletion of 0.6 to 1.2 mg/L.

8.1.8 Dilution water Quality Control (QC) – Unseeded Blank

1) Siphon 300 mL of dilution water into BOD bottle.
2) Take initial DO and record in bench sheet.
3) Replace any displaced dilution water, stopper, cap, and incubate at 20 ± 1^o C for 5 days.
4) The 5-day DO should be ± 0.2 mg/L of initial DO.

8.1.9 BOD standard preparation - Quality Control (QC) check

1) Obtain Glucose-Glutamic Acid BOD standard from the "Micro Refrigerator" located under the fecal incubators.
2) Allow the standard to warm up to room temperature before using.
3) Using a sterile 10 mL pipette, add 6 mL BOD standard into BOD bottle labeled "*Standard"*.
4) Siphon dilution water into BOD bottle until full.
5) Take initial DO and record in bench sheet.
6) Replace any displaced dilution water, stopper, cap, and incubate at 20 ± 1^o C for 5 days.
7) The 5-day BOD of the standard should be 198 mg/L ± 30.5 mg/L.

8.1.10 Sample preparation

1) Place the sample volume indicated on the bench sheet into each labeled sample BOD bottle.
2) Add 4 mL of BOD seed into each bottle.
3) Siphon dilution water into BOD bottle until full.
4) Read initial DO and record each sample on bench sheet.
5) Replace lost volume of BOD bottle with dilution water until full and cap with stopper.
6) Place plastic caps over BOD bottles to protect water seal.
7) Rinse probe with lab water between samples.
8) Incubate BOD bottles at 20 ± 1º C for 5 days.
   Check incubator temperature and adjust if needed. For each incubator, record in the daily logs both the date and daily temperatures, and initial. The daily logs are located next to BOD station.

8.1.11 After 5-day incubation

1) Calibrate DO meter (see SOP-DO, Section 8).
2) Measure and record the 5-day DO values for each sample on the bench sheet and turn in for data entry (see Section 11. Data Analysis and Calculations).

8.2 Helpful Hints

1) Samples that are caustic or acidic should be neutralized to pH 6.5 to 7.5.
2) Samples containing residual chlorine should have chlorine removed by adding 10 drops of 10% Sodium sulfite (Na_2SO_3) solution.
3) For samples that are known to have high concentrations of solids, make serial dilutions in 100 mL volumetric flasks using Nanopure laboratory water so as to yield a 40% to 60% DO uptake after 5 days.

9. QA/QC Requirements

9.1 Calibrate DO meter at the beginning of each analysis day. Recalibrate in the afternoon.
9.2 Run BOD Standard each batch: 6 mL of 198 mg/L GGA standard.
9.3 Run 4 blanks with each batch (3 seeded, one unseeded).
9.4 Run 1 random duplicate per 20 samples.
9.5 Data acceptance criteria:
   Analysis values for GGA Standards should be 198 mg/L ± 30.5 mg/L.
   Duplicate values must agree within 5%.
   Blank depletion values must be no greater than 0.2 mg/L.
If these criteria are not met, corrective action is indicated (See Quality Assurance Program (QAP) Sec. 15 “Corrective Action Policies and Procedures”).

10. Expected Results

10.1 KPDES Permit Requirements

PTE: 10 mg/L maximum for monthly average and
15 mg/L maximum for weekly average.

In the event that analysis results indicate values greater then KPDES permit requirements, notify the Plant Superintendent and Laboratory Supervisor.

10.2 Process Ranges

Influent BOD values vary with season, rainfall amounts and influent flow rate. Typical influent values for Town Branch Plant range 200 mg/L to 50 mg/L with an average of approximately 130 mg/L.
West Hickman Plant influent values range from 250 mg/L to 50 mg/L with an average of approximately 180 mg/L.
Typical effluent BOD values at both Town Branch and West Hickman are <10 mg/L.

11. Data Analysis and Calculations

Initial and final dissolved oxygen values are entered into the BOD/CBOD worksheet which calculates the BOD or CBOD values in accordance with the following

Given:

B_1 = initial DO reading of blank
B_2 = initial DO reading of blank
D_1 = initial DO reading of sample
D_2 = 5-day DO reading of sample
S = volume of seed used in blank
P = volume of seed per BOD sample bottle
V = volume of sample in BOD bottle

Then by Calculation:

$(B_1 - B_2)$ = depletion of blank
$(D_1 - D_2)$ = depletion of sample
$(B_1 - B_2 /S)$ = DO used/ml of seed
F = seed correction = $(B_1 - B_2)/S*P)$
C = $(D_1 - D_2)$-F = depletion corrected for seed
BOD or CBOD = C*(300/V)

12. Bibliography

12.1 U.S. EPA Method 405.1, Biochemical Oxygen Demand, 5 Days @ 20ºC, Issued 1971, Editorial revision 1974. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

12.2 Standard Methods 5210-A. and 5210-B. Biochemical Oxygen Demand (BOD). APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

Total Residual Chlorine Analysis
HACH AutoCAT 9000 Total Chlorine Amperometric Forward Titration
procedure equivalent to
EPA Method 330.3 Chlorine, Total Residual (Titrimetric, Amperometric), Issued 1978
Standard Methods Part 4500-Cl D. (Chlorine Residual Amperometric Titration Method)

1. Scope, Significance to Process and Application

1.1 Disinfection by chlorination is considered to be the primary mechanism for the inactivation/destruction of pathogenic organisms in wastewater treatment plant effluents and to prevent the spread of waterborne diseases to downstream users and the environment. Final clarifier effluent is treated with chlorine as enters the chlorine contact tanks allowing contact time for disinfection to transpire. Final effluent is then treated with a dechlorinating agent reducing chlorine residual concentration to within acceptable limits (see Section 10.1 Permit Limits). Complete dechlorination is necessary to prevent chlorine related adverse effects on the receiving environment. Town Branch Waste Water Treatment Plant uses Chlorine Dioxide (ClO_2) for chlorination and Sulfur Dioxide (SO_2) as the dechlorinating agent. Residual Chlorine analysis of treated plant effluent validates efficacy of dechlorinating agent dosing and permit compliance.

2. Summary of Method

2.1 Executive Summary

Town Branch Laboratory uses a HACH AutoCAT 9000 autotitrator to perform Residual Chlorine determinations. The AutoCAT 9000 bench top system automatically completes all USEPA- approved amperometric titration methods for chlorine, calculates analyte concentration, and provides real-time graphics display. The AutoCAT's forward amperometric titration procedure has a range of 0.0012 mg/L to 5.0 mg/L with an estimated detection limit of 0.0012 mg/L

2.2 Discussion

Chlorine (hypochlorite ion, hypochlorous acid) and chloramines liberate iodine from potassium iodide at pH 4 or less in stoichiometeric proportions. The iodine is titrated with a reducing agent phenylarsine and an amperometer detects the endpoint. Although the actual measurement is that of the samples oxidation potential, it is calculated and expressed as mg/L Cl because chlorine is the dominating oxidizing agent present.

3. Health & Safety Precautions

3.1 Glassware involved, possible cut hazard.
3.2 Wastewater samples have the potential to be hazardous, use appropriate caution.

4. Sample Handling and Preservation

4.1 Residual chlorine is subject to dissipation by exposure to sunlight, mechanical agitation, exchange of gases with the atmosphere and reaction with compounds in the wastewater over time. For these reasons chlorine residuals should be analyzed immediately (within 15 minutes of sampling time).

4.2 The sample should be taken gently into a glass 300 mL BOD bottle, completely filling to above the base of the neck and installing the tapered glass stopper in a manner that precludes air bubbles in the sample.

4.3 All glassware used in this method must have no chlorine demand, therefore do not use plastic containers and pre-treat glassware accordingly. To remove chlorine demand from clean glassware, soak in a dilute bleach solution (1 mL commercial bleach to 1 liter of Nanopure water) for at least one hour. After soaking, rinse thoroughly with Nanopure water. After analysis, thoroughly rinse all glassware with Nanopure water to reduce the need for pretreatment.

5. Reagents

5.1 Reagent 1 - Potassium Iodide 5%

5.2 Reagent 2 - pH 4.00 Buffer (Certified Acetic Acid). Both reagents are located adjacent to the AutoCAT unit

5.4 Phenylarsine Oxide Standard Solution 0.00564 N

5.5 Chlorine Standard Solution, 25-30 mg/L as Cl_2

6. Equipment & Lab Ware

6.1 HACH AutoCAT 9000 - Chlorine Amperometric Titrator

6.2 Beakers 250 mL

6.3 Graduated Cylinders 250 mL

6.4 1 mL fixed volume Finnpipette and 1 mL tips

6.5 Stirring bars.

7. Interferences

7.1 Accurate determinations of free chlorine cannot be made in the presence of Nitrogen trichloride or Chlorine dioxide.

7.2 Some organic chloramines can also interfere.

7.3 Free halogens other than chlorine also will titrate as free chlorine.

7.4 Interference from copper has been noted in samples taken from copper pipe or after heavy copper sulfate treatment of reservoirs.

7.5 Contamination of probe by metal ions such as copper, silver, iron interfere with amperometric titrations. Fouled electrodes will not produce sharp endpoints.

7.6 Extended sample hold times, volatilization from mechanical agitation, and exposure to various light sources can affect results.

7.7 At very low temperatures, there is slow response of cell and longer time is required, but precision is not compromised.

8. Procedures

8.1 Steps

1) Prior to testing, pre-rinse all glassware and stir bars with sample (Do not rinse with Nanopure once pre-rinsed).
2) Using a 250 mL graduated cylinder measure 200 mL of sample.
3) Pour sample into a 250 mL beaker with stirring bar, raise the electrode assembly and place the beaker on unit.
4) Turn on instrument. The display will request user to press "1" for "Total Cl_2 Fwd", press "1", display will then request user confirmation, press "1" to confirm.
5) The display will request confirmation of sample volume (200 mL) press "1"
6) The sample will begin to stir.
7) Display will request the addition of 1 mL of Potassium Iodide 5% (Reagent 1), pipette reagent into sample, then press "OK"
8) Display will request the addition of 1 mL of Acetate buffer pH 4 (Reagent 2), pipette reagent into sample, then press "OK"
9) A mixing timer will countdown for 5 sec. then the display will request that the electrodes be dipped into the sample, lower electrode assembly into sample and press "OK".
10) The display will request user to confirm the "Increment Setting" (should be 0.0010), press "1" to confirm.
11) Unit will begin analysis; total time required for analysis will vary with sample strength and chosen increment value. During analysis the display graphs the progress of the titration. Upon completion the unit will display the resulting concentration value and calculated confidence limits, press "OK" to accept results.
12) Record the results on the Total Chlorine Residual bench sheet. In the case of the PTE sample, also log (in the provided location) the time sampled, time received, and time analysis began. Note: If sample hold time (time sampled to time analysis begins) exceeds 15 minutes the analysis is void and must be rerun, beginning with resampling.
13) Select "END" if done with analysis or "Continue" to proceed to the next sample to be analyzed.

Note: More detailed general information on the AutoCAT unit can be found in the operator's manual with details on the Forward Amperometric procedure starting on page 101. The manual is located on the shelf adjacent to the AutoCAT unit.

8.2 Chlorine Standard Analysis

1) Obtain a Chlorine Standard Solution (25-30 mg/L) ampoule from "Chemical Storage" fridge.
2) Carefully break top off ampoule.
3) Pipette 1.0 mL of standard into 1000 mL volumetric flask.
4) Bring to 1000 mL with Nanopure water.
5) Measure 200 mL of standard solution into beaker with stir bar.
6) Run titration as indicated in Section 8.1.
7) Record results in bench sheet.
8) The Laboratory Supervisor will determine if the standard is within the expected range.
9) Measure 200 mL of Nanopure water into a clean 250 mL beaker and analyze as indicated in Section 8.1 (This will be a Blank to confirm no chlorine carry-over). Record results in bench sheet.
10) If chlorine is detected, re-run Blank until Below Detection Limit (BDL) is obtained.

8.3 Helpful Hints

1) Analysis results are directly proportional to sample volumes therefore it is very important that sample volume measurement is accurate.
2) Clean conditioned electrodes are required for the production of sharp, well defined endpoints that are needed for precise analysis. Rinse electrodes thoroughly before and after each use with Nanopure water, and store in Nanopure water.
3) Routine use of the "Electrode Cleaning and Conditioning" procedure as described in Section 9.1.4 of the Operator's Manual will prevent problems.
4) Glassware must be clean and free of chlorine demand see section 4.3

9. QA/QC Requirements

9.1 A diluted standard (25-30 mg/L) and Blank(s) must be run once a week (See Section 8.2).

9.2 5% of all samples must be run in duplicate.

9.3 Data acceptance criteria:

1) Results for the Standard must agree within 10% of the standard's known value.
2) Duplicate values must agree within 5%.
3) If these criteria are not met, corrective action is indicated. See Quality Assurance Program (QAP) Sec. 15 "Corrective Action Policies and Procedures".

10. Expected Results

10.1 KPDES Permit Requirements
KPDES Permit Limits on plant effluent residual chlorine at Town Branch WWTP is a maximum monthly average of 0.010 mg/L, with a daily maximum limitation of 0.019 mg/L. In the event that analysis results indicate values greater then KPDES permit requirements, retest. If the value indicated by the retest is greater than KPDES permit requirements, **Immediately** notify the Plant Superintendent and Laboratory Supervisor.

10.2 Process Ranges
Expected residual chlorine results on plant effluent samples will be less than 0.010 mg/L, typically the results are BDL (below detection limit).

11. Data Analysis and Calculations

11.1 Concentration values are read directly from the AutoCAT unit's display, all calculations are preformed internally.
11.2 The Laboratory Supervisor will determine if the results for the standard are within the expected range.

12. Bibliography

12.1 U.S. EPA Method 330.3 Chlorine, Total Residual (Titrimetric, Amperometric) Issued 1978. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

12.2 Standard Methods 4500-Cl D. Chlorine Residual Amperometric Titration Method. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

12.3 HACH AutoCat 9000 Chlorine Amperometric Titrator Instruction Manual. HACH Company, Loveland, CO.

Free Cyanide CN (F)
HACH Method 8027 Pyridine-Pyrazalone Method

1. Scope, Significance to Process and Application

1.1 Measurement of the concentration of free cyanide in industrial wastewater samples.

2. Summary of Method

2.1 Discussion
The Pyridine-Pyrazalone method used for measuring cyanide gives an intense blue color with free cyanide.

3. Health & Safety Precautions

3.1 All municipal and industrial wastewaters are potentially hazardous. Gloves and safety glasses should be worn when dispensing these samples.
3.2 Cyanides, their solutions, and Hydrogen cyanide liberated by acids are very poisonous. Both gas and solutions can be absorbed through the skin. Latex gloves and safety glasses should be used.
3.3 CyaniVer 3, CyaniVer 4, and CyaniVer 5 reagent powder pillows are used. May be respiratory hazard.

4. Sample Handling and Preservation

4.1 Collect samples in glass or plastic bottles and analyze as quickly as possible. The presence of oxidizing agents, sulfides and fatty acids can cause the loss of cyanide during sample storage. Samples containing these substances must be pretreated as described in the following procedures before preservation with sodium hydroxide. If the sample contains sulfide and is not pretreated, it must be analyzed within 24 hours.

5. Reagents

5.1 CyaniVer 3, CyaniVer 4, and CyaniVer 5 reagent powder pillows.

6. Equipment & Lab Ware

6.1 Plastic beakers, at least 50 mL
6.2 Funnel, plastic or glass
6.3 Glass Microfibre Filter paper, 125mm diameter

6.4 Two 1-inch sample cells, 10-mL with cap (zeroing vial/sample vial)
6.5 Fixed 5 mL pipette with 5 mL pipette tip
6.6 Spec Color Standards kit
6.7 DR/4000 1-inch Cell Adapter
6.8 HACH DR/4000 Spectrophotometer

7. Interferences

7.1 Chlorine.
7.2 Metals.
7.3 Oxidizing agents.
7.4 Reducing agents.
7.5 Turbidity

8. Procedures

8.1 Steps

8.1.1 Filtration

1) Pour approximately 40 mL from the Cyanide sample container to the plastic beaker as soon as the sample arrives. Preserve the remaining sample with 1.25 mL of 12.5 N NaOH for total cyanide analysis.
2) Fold the glass microfibre filter paper to fit in the funnel.
3) Slowly pour sample into filter so it does not overflow.
4) Collect filtered sample.

8.1.2 Reaction

1) Fill sample cell with 10 mL of filtered sample.
2) Add CyaniVer 3 powder pillow to the 10 mL in sample cell, cap and shake 30 seconds. Wait an additional 30 seconds leaving the sample undisturbed.
3) Add CyaniVer 4 powder pillow. Shake for 10 seconds and immediately proceed to next step.
4) Immediately add CyaniVer 5 powder pillow. Shake the cell vigorously for 15 seconds.
5) Set timer for 30 minutes.
6) If there is any cyanide present the sample will turn blue.

8.1.3 Using the DR4000 spectrophotometer on positive reaction

1) Fill a round sample cell with 10 mL of filtrated sample; this will be used as the BLANK.
2) Touch HACH Programs on keypad and select program “1750 Cyanide”.

3) Place BLANK sample into DR4000 cell holder. Close the light shield. Touch "Zero" to zero instrument.
4) Place the prepared sample into the cell holder. Close the light shield. Results will appear as mg/L cyanide.

8.2 Helpful Hints

1) Make sure that the CyaniVer 5 powder pillow is added immediately after the 10 second shaking period from the previous reagent.
2) If interference is present, the sample will turn into a cloudy, murky solution.

9. QA/QC Requirements

9.1 Use free cyanide standards.

10. Expected Results

Every now and then, a positive reaction occurs. If it is higher than the limit posted on the industrial waste laboratory report (red number), inform the Laboratory Supervisor immediately.

10.1 KPDES Permit Requirements

Depends on the particular industry being sampled.

11. Data Analysis and Calculations

11.1 None required.

12. Bibliography

12.1 HACH DR/4000 Method 8027. HACH Company, Loveland, CO.

Total Cyanide CN (T)
HACH Method 8027 Pyridine-Pyrazalone Method with Distillation
Lachat Instruments Method 10-204-00-1-X (MICRO DIST Cyanide-1)

1. Scope, Significance to Process and Application

1.1 Measurement of the concentration of total cyanide in industrial waste water samples.

2. Summary of Method

2.1 Discussion

The Pyridine-Pyrazalone method used for measuring cyanide gives an intense blue color with free cyanide.

3. Health & Safety Precautions

3.1 All municipal and industrial wastewaters are potentially hazardous. Gloves and safety glasses should be worn when dispensing these samples.

3.2 Cyanides, their solutions, and Hydrogen cyanide liberated by acids are very poisonous. Both gas and solutions can be absorbed through the skin. Latex gloves and safety glasses should be used.

3.3 Industrial waste samples, CyaniVer 3, CyaniVer 4, and CyaniVer 5 reagent powder pillows are used. Also used during preparation for the distillation process is 0.75 mL of 7.11M sulfuric acid/0.79M magnesium chloride solution. Latex gloves and safety glasses should be used.

3.4 During the distillation process the use of heat resistant gloves and safety glasses are required.

4. Sample Handling and Preservation

4.1 Collect samples in glass or plastic bottles. Preserve the sample with 1.25 mL of 12.5N NaOH for analysis within 14 days of collection.

5. Reagents

5.1 CyaniVer 3, CyaniVer 4, and CyaniVer 5 reagent powder pillows
5.2 Releasing agent (7.11M sulfuric acid/0.79M magnesium chloride solution)
5.3 Trapping solution (0.950M standardized NaOH)
5.4 2.5N HCl

6. Equipment & Lab Ware

6.1 Lachat Micro Dist heating block
6.2 Lachat Micro Dist collector tubes, membranes and caps
6.3 Seal press
6.4 1-inch sample cells for as many sample as being analyzed
6.5 Fixed 0.580 mL pipette and tip
6.6 Adjustable 3.00 mL pipette and tip
6.7 Squirt bottle with Nanopure water
6.8 0.75 mL and 1.59 mL repipetor bottles
6.9 Spec Color Standards kit
6.10 HACH DR/4000 1-inch Cell Adapter
6.11 HACH DR/4000 Spectrophotometer

7. Interferences

7.1 Chlorine.
7.2 Metals.
7.3 Oxidizing agents.
7.4 Reducing agents.
7.5 Turbidity

8. Procedures

8.1 Steps

8.1.1 Distillation

1) Allow Micro Dist heater block to warm up to 120^{o} C.
2) Add 1.59 mL of trapping solution in each collector tube.
3) Cap tube with membrane.
4) Add 6 mL of sample in sample tube and add 0.75 mL releasing agent (0.95M NaOH).
5) Immediately place the sample tube in the seal press. Seal the collector tube to the sample tube using the pressing motion of the seal press.
6) Place tubes on the preheated block and set timer for 30 minutes.
7) When time is up remove the tube from the heating block and immediately pull of the sample tube using a downward, twisting motion. Allow 15 minutes for tubes to cool.
8) Rinse the walls of the collector tube. Slowly return the collector tube to an upright position to gather all the droplets.
9) Break away the top half of the collector tube. Dilute to the 6 mL mark with Nanopure water.
10) The distilled 6 mL sample in now ready to be analyzed.

8.1.2 Reaction

1) Fill sample cell with the 6 mL distilled sample.
2) Add 0.580 mL of 2.5N HCl to sample cell, cap and shake for 10 seconds.
3) Add CyaniVer 3 powder pillow to the 10 mL in sample cell, cap and shake for 30 seconds. Wait an additional 30 seconds leaving the sample undisturbed.
4) Add CyaniVer 4 powder pillow. Shake for 10 seconds and immediately proceed to next step.
5) Immediately add CyaniVer 5 powder pillow. Shake the cell vigorously for 15 seconds.
6) Set timer for 30 minutes.
7) If there is any cyanide present the sample will turn blue.

8.1.3 Using DR4000 spectrophotometer on positive reaction

1) Use Spec Color Standards kit to test and zero the instrument.
2) Touch "HACH Programs" on DR4000 keypad and select program "1750 Cyanide".
3) Place BLANK sample into cell holder. Close the light shield. Touch "Zero" to zero instrument.
4) Place the prepared sample into the cell holder. Close the light shield. Results will appear as mg/L cyanide.

8.2 Helpful Hints

1) Make sure that the CyaniVer 5 powder pillow is added immediately after the 10 second shaking period from the previous reagent.
2) If interference is present the sample will turn into a cloudy, murky solution.

9. QA/QC Requirements

9.1 Use total cyanide standards.

10. Expected Results

Every now and then, a positive reaction occurs. If it is higher than the limit posted on the industrial waste laboratory report (red number), inform the Laboratory Supervisor immediately.

10.1 KPDES Permit Requirements

Depends on the particular industry being sampled.

11. Data Analysis and Calculations

11.1 None required.

12. Bibliography

12.1 HACH DR/4000 Method 8027. HACH Company, Loveland, CO.

12.2 Lachat Micro Dist User Manual, Method Cyanide in Waters (MICRO DIST Cyanide-1). Lachat Instruments, HACH Company, Loveland, CO.

12.3 U.S. EPA. Method 335.4. Revision 1.0, August 1993. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

Chromium, Total and Hexavalent (Cr^{6+})
TNT+ HACH Method 10219 (Chromium, Total)
TNT +HACH Method 10218 (Chromium, Hexavalent)
1, 5-Diphenylcarbohydrazide Method (0.03 to 1.00 mg/L Cr)

1. Scope, Significance to Process and Application

1.1 Measurement of the concentration of total and hexavalent (Cr^{6+}) in industrial wastewater samples.

2. Summary of Method

2.1 Executive Summary

Hexavalent chromium, if present in industrial wastewater samples, is measured by HACH DR5000 spectrophotometer. Hexavalent chromium enters the water from industrial wastes from metal plating facilities and from cooling towers where chromate is used to inhibit corrosion.

2.2 Discussion

In the total chromium procedure, all chromium in the sample is oxidized to the hexavalent chromium (Cr^{6+}). The hexavalent chromium then reacts with 1,5-diphenylcarbazide to form 1,5-diphenylcarbazone. The amount of red color formed with hexavalent chromium is directly proportional to the amount of chromium present in the sample. Determine trivalent chromium by subtracting the results of a separate hexavalent chromium test from the results of the total chromium test. Test results are measured at 543 nm.

3. Health & Safety Precautions

3.1 All municipal and industrial wastewaters are potentially hazardous. Gloves and safety glasses should be worn when dispensing these samples.

3.2 Watch out for broken glass.

4. Sample Handling and Preservation

4.1 Collect samples in acid-washed glass or plastic containers.

4.2 To preserve samples for total chromium analysis, adjust the pH to 2 or less with nitric acid (approximately 2 mL/L of the acid). Store preserved samples at 4 °C for up to 6 months. Bring the sample temperature to 15–35 °C adjust the pH to about 4 with 5.0 N NaOH before analysis.

4.3 To preserve samples for hexavalent chromium analysis, adjust the pH to 8 with 1N NaOH. Store at 4 °C for up to 24 hours. Bring sample to 15–35 °C. No pH neutralization is required.

5. Reagents

5.1 Chromium, Total and Hexavalent TNTplus Reagent Set (HACH TNT854)
5.2 Chromium, Trivalent, Standard Solution (50 mg/L Cr^{3+})
5.3 Chromium, Hexavalent Standard Solution (50 mg/L Cr^{6+})
5.4 Sodium Hydroxide, 1.0 N and 5.0 N

6. Equipment & Lab Ware

6.1 HACH DRB200 Reactor, 9x13 mm
6.2 Test Tube Rack
6.3 Variable volume pipette (1–5 mL) and tips
6.4 Plastic beakers or medicine cups
6.5 TNTplus Reactor Adapter Sleeves, 16-mm to 13-mm diameter
6.6 Funnel, plastic or glass
6.7 Glass Microfibre Filter paper, 125mm diameter
6.8 HACH DR5000 spectrophotometer

7. Interferences

7.1 The ions listed in *Interfering substances* (See HACH method) have been individually checked up to the given concentrations and do not cause interference. Cumulative effects and the influence of other ions have not been determined.
7.2 Larger amounts of iron, copper and reducing and oxidizing agents give low-bias results. Lead, mercury and tin give high-bias results.
7.3 ***Important Note:*** *Undissolved chromium is not determined with the determination of chromium(VI). An analyte concentration greatly (above 20 mg/L) in excess of the stated range will adversely affect color formation, resulting in a false reading within the method range.*
7.4 Measurement results can be verified using sample dilutions or standard additions.

8. Procedures

8.1 Steps
8.1.1 Filtration
1) Pour the sample from the sample container to the plastic beaker as soon as the sample arrives.
2) Fold a glass microfibre paper to fit in the funnel.
3) Slowly pour sample into funnel so it does not overflow.
4) Collect filtrate.

8.1.2 **Method 10219 for Total Chromium**

1) Turn on the DRB200 Reactor. Preheat to 100 °C. For DRB200 Reactors with 16-mm wells, make sure the 16-mm to 13-mm adapter sleeve have been placed into each well before turning on the reactor.
2) Carefully remove the protective foil lid from the DosiCap™ *Zip*. Unscrew the cap from the vial.
3) Pipet 2.0 mL of sample into the vial.
4) Turn the DosiCap *Zip* over so that the reagent side faces the vial. Screw the cap tightly onto the vial.
5) Shake the capped vial 2–3 times to dissolve the reagent in the cap. Make sure that the reagent has dissolved by looking down through the open end of the DosiCap.
6) Heat the vial for one hour at 100 °C.
7) When the timer expires remove the hot vial from the reactor. Cool the vials to 15–35 °C. **Do not invert** the vial after digestion.
8) Screw an orange DosiCap B onto the cooled vial.
9) Invert the vial 2-3 times to mix.
10) After inverting the tube, allow the vial to sit undisturbed for 2–3 minutes.
11) After the timer expires, invert the vial again 2–3 times.
12) Thoroughly clean the outside of the vial with a Kim-wipe. Insert the prepared vial into the DR5000 cell holder. The instrument reads the barcode, then selects and performs the correct test. Results are in mg/L Cr.
13) No instrument Zero is required.

8.1.3 **Method 10218 for Hexavalent Chromium**

1) Remove cap and pipet 2.0 mL of sample into the vial.
2) Screw an orange DosiCap B on the vial.
3) Invert the vial 2-3 times to mix.
4) After inverting the tube, allow the vial to sit undisturbed for 2–3 minutes.
5) After the timer expires, invert the vial again 2–3 times.
6) Thoroughly clean the outside of the vial with a Kim-wipe.
7) Insert the prepared vial into the DR5000 cell holder. The instrument reads the barcode, then selects and performs the correct test. Results are in mg/L Cr^{6+}.
8) No instrument Zero is required.

8.1.4 **Reagent blanks**

1) A reagent blank can be measured and the value subtracted from the results of each test performed using the same reagent lot number. Use deionized water in place of sample and run the procedure as described.
2) To subtract the value of the blank from a series of measurements, measure the blank per step 12 of the total chromium procedure or step 7 of the hexavalent chromium procedure.
3) Press **OPTIONS**>**MORE**>**REAGENT BLANK**. Press **ON**. The measured value of the blank should be displayed in the highlighted box. Press **OK** to accept this value. The reagent blank value will now be subtracted from all results until the function is turned off or a different method is selected.
4) Alternately, the blank can be recorded and entered at any later time by pressing the highlighted box and using the keypad to enter the value.

8.1.5 **Sample blanks**

1) Colored or turbid samples can cause high results. The digestion in the total chromium procedure usually destroys all color and turbidity and a sample blank is not required. To compensate for color or turbidity in the determination of hexavalent chromium, the procedure is repeated and the color forming reagent that is present in the DosiCap B is not added.
2) To determine the sample blank for hexavalent chromium:
   a) Run the procedure as written, but do not add the DosiCap B Reagent in step 2.
   b) Cap the vial with the original DosiCap *Zip* (do not remove the foil).
   c) The value obtained in step 7 is subtracted from the value obtained on the original hexavalent chromium sample to give the corrected sample concentration.
   d) Alternatively, hexavalent chromium samples that contain turbidity only may be filtered through a membrane filter and analyzed using the hexavalent procedure. Results are reported as dissolved hexavalent chromium.
3) Samples without color or turbidity do not require sample blanks.

8.2 Helpful Hints

1) Make sure to correct the test results for volume dilutions.
2) Wipe off any liquid or fingerprints from TNT+ tube.

9. QA/QC Requirements

9.1 None required.

10. Expected Results

10.1 Report positive reactions to supervisor immediately.

10.2 KPDES Permit Requirements
Depends on the particular industry being sampled.

11. Data Analysis and Calculations

11.1 None required.

12. Bibliography

12.1 HACH Water Analysis Handbook. Method 8023, Chromium Hexavalent (1,5-Diphenylcarbohydrazide Method). 2nd Edition. 1992. HACH Company, Loveland, CO.

12.2 HACH. DOC316.53.01035. Chromium, Total and Hexavalent. 1,5-Diphenylcarbohydrazide, Method 10218 (Chromium, Hexavalent) and Method 10219 (Chromium, Total), TNTplus™ 854. 2008. HACH Company, Loveland, CO.

12.3 Standard Methods Part 3500 B. Chromium, Colorimetric Method. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

Dissolved Oxygen Analysis (D.O.)
HACH Method 10360 Luminescent Dissolved Oxygen Probe Method
Proposed EPA Method 360.3 (Luminescence) for the Measurement of Dissolved Oxygen in Water and Wastewater

1. Scope, Significance to Process and Application

1.1. The Dissolved Oxygen (D.O.) analysis is the measurement of the concentration of oxygen dissolved in a water sample.
1.2. This method is recommended for samples containing intense color or turbidity.
1.3. This method is recommended for work in the field, as the equipment is portable, allowing hold times to be minimized.
1.4. KPDES Permit Limits on Plant Effluent is a minimum of 7.0 mg/L.
1.5. Dissolved Oxygen concentration levels are very important in both process plant effluent. In process, dissolved oxygen is required by various organisms and the plant effluent dissolved oxygen levels must be conducive to the receiving environment and within permit limits (See Section 10.1).

2. Summary of Method

2.1 Executive Summary

Dissolved Oxygen is measured directly by a HACH model HQ40d portable meter and HACH model LBOD101 probe, located on the BOD bench. After the meter indicates a stable reading the analyst records the value.

2.2 Discussion

The HACH LDO system uses a sensor coated with a luminescent material. Blue light from an LED is transmitted onto the sensor surface, exciting the luminescent material, which then emits red light as it relaxes. The presence of DO in the process shortens the time it takes for the red light to be emitted. By measuring the time lapse between when the blue light was transmitted and the red light is emitted, a correlation is made to the concentration of DO in the effluent or other solution. Between measurements, a red LED is used as an internal reference. The measurement range for the method is 0.02 - 20.0 mg/L. The Method Detection Limit (MDL; 40 CFR 136, Appendix B) has been determined as 0.05 mg/L and the Minimum Level (ML; Reference 15.4) has been set at 0.20 mg/L.

3. Health & Safety Precautions

3.1 Glassware involved: possible cut hazard.
3.2 All municipal and industrial wastewaters are potentially hazardous.
Gloves and safety glasses should be worn when dispensing these samples.

4. Sample Handling and Preservation

4.1 Sample must be collected in a glass bottle (BOD bottle with stopper) filled to top, with no airspace.
4.2 Sample must be analyzed immediately (15 minutes maximum on permit samples).
4.3 There is no applicable preservative with this method.

5. Reagents

5.1 Nanopure Lab Water

6. Equipment & Lab Ware

6.1 HACH HQ40d portable multi-meter
6.2 HACH Model LBOD101-01 probe with integrated stirrer
6.3 BOD bottles with 300 mL capacity and tapered ground glass stoppers
6.4 Sensor Cap replacements (HACH part # 5838000)
6.5 LDO Stirrer replacement kit (HACH part # 5850800)

7. Interferences

7.1 Salinity (salinity correction available, See Section 8.4.3 of the Users Manual).
7.2 Reactive gas: chlorine and hydrogen sulfide.
7.3 Air bubbles in sample or on surface of probe tip.

8. Procedures

8.1 Steps

8.1.1 Calibration and Start Up

It is recommended that the HQ40d Users Manual be initially consulted when following these procedures. The manual is located in a yellow folder labeled “Lab D.O. Meter”, in the yellow bin located adjacent to the meter.

1) Press the power button and allow the unit to perform its startup self check routine.

2) Clean by rinsing with Nanopure lab water, then gently blot dry the probes tip with a Kim-Wipe. Inspect the probe tip for indications contamination or damage.

3) Take a 300 mL BOD bottle containing approximately one inch of lab water, stopper and shake, remove stopper and replace it with the probe.

4) Press Calibrate (blue button), the meter will prompt you to "Dry the probe and place in water saturated air & press "Read". Press the "Read" button, the screen will scroll from 0 to 100%, then indicate "Calibration Complete". Record from display screen both the temperature and the dissolved oxygen value (indicated under the temperature). Log the values on the dissolved oxygen calibration section of the Dissolved Oxygen bench sheet under Temperature and Dissolved Oxygen from HQ40d.

5) Note the barometric pressure value from the laboratory barometer (located adjacent to the D.O. meter) and record on the dissolved oxygen calibration section of the Dissolved Oxygen Bench sheet under "Barometer Reading".

6) On the Lab computer, open the excel spreadsheet entitled "DO Meter Calibration Sheet" and enter the barometric pressure, temperature and dissolved oxygen values from the dissolved oxygen calibration section of the Dissolved Oxygen Bench sheet. The spreadsheet will calculate the "Dissolved Oxygen Calibration Point", the "Dissolved Oxygen @ 1 ATM" and the Slope %. Transfer the three values onto the dissolved oxygen calibration section of the Dissolved Oxygen bench sheet, then print a copy of the spreadsheet and file it in the yellow file (located adjacent to the D.O. meter).

7) Note the difference between the Dissolved Oxygen from HQ40d and the Dissolved Oxygen Calibration Point - if it is greater than 0.2 mg/L, then the calibration is not acceptable and must be repeated until criteria is met.

8) If sample measurements are made in the afternoon, the meter must be calibrated again, due to changes in atmospheric pressure. Repeat steps in Section 8.1.1.

8.1.2 Measurements

1) Make sure that the meter is properly calibrated.

2) Rinse the LBOD probe tip with Nanopure water.

3) Place the probe into BOD bottle, filled to the base of its neck with sample, and turn on the self-contained stirring unit switch located on top of the probe. Assure that there are no air bubbles on the surface of the probe tip.

4) Press "Read", the screen will display "Stabilizing" and a progress bar will scroll from 0 to 100%. Reading stability is indicated by the appearance of a "Padlock" icon in the upper left corner of the display screen.

5) Record the indicated value, turn the stirrer off, remove the probe and rinse tip with Nanopure water and proceed to the next sample or store until needed.

6) DO NOT store probe in the BOD bottle containing water. Probe can be stored dry on the bench top.

8.2 Helpful Hints

1) The meter is designed to be maintenance free, when needed, clean the exterior with a damp cloth.
2) The probe's maintenance consist of maintaining the probe tip clean, frequent rinsing with Nanopure water is sufficient.
3) DO NOT scrub the sensor cap or lens.
4) DO NOT use any organic solvents on the sensor cap or probe body.

9. QA/QC Requirements

9.1 Meter must be calibrated a minimum of once per analysis day.
9.2 Permit sample hold times must be 15 minutes or less.
9.3 Probe condition must be properly maintained through routine cleaning (See Section 8.2, Helpful Hints).

10. Expected Results

10.1 KPDES Permit Requirements

7.0 mg/L is the lowest D.O. value allowable in a Plant Effluent sample at any given time. In the event of a indicated value less than 7.0 mg/L, assure correct calibration, resample, and retest. If the value indicated by retest is less than 7.0 mg/L. **Immediately** notify the Plant Superintendent and Laboratory Supervisor.

10.2 Process Ranges

Raw influent dissolved oxygen values are typically less than 1 mg/L.
Target values for mixed liquor dissolved oxygen concentration in the aeration basins is 2.0 mg/L.

11. Data Analysis and Calculations

11.1 None required, values are taken directly when measurement stability is indicated.

12. Bibliography

12.1 Report on the Validation of Proposed EPA Method 360.3 (Luminescence) for the Measurement of Dissolved Oxygen in Water and Wastewater. August 2004. HACH Company, Loveland, CO.

12.2 Memorandum: EPA Recommendation for the use of HACH method 10360 [Revision 1.1, January 2006] (ATP Case # N04-0013).

12.3 HACH HQ Series Portable Meter Users Manual, September 2006, Edition 5. HACH Company, Loveland, CO.

Fecal Coliform - Membrane Filter Procedure
U.S. EPA 600/8-78-017 Microbiological Methods for Monitoring the Environment: Water and Wastes

1. Scope, Significance to Process and Application

1.1 The fecal coliform analysis is applicable to investigations of stream pollution, raw water sources, and wastewater treatment systems.

1.2 The fecal coliform analysis differentiates between coliforms of fecal origin.

2. Summary of Method

2.1 Executive Summary

The sample is filtered through a Millipore® membrane filter. The filter is placed on a filter pad containing media in a sterile Petri dish. The samples are then incubated at 44.5°C ± 0.2°C for 24 hours ± 2 hours. Colonies are counted and fecal coliform calculations are performed.

2.2 Discussion

Fecal coliforms are defined as gram-negative, non-spore forming rods. The major species is *Escherichia coli*, which indicates fecal pollution and the presence of enteric pathogens. Colonies produced by fecal coliform bacteria are various shades of blue. Non-fecal coliform colonies are gray to cream colored.

3. Health & Safety Precautions

3.1 All municipal and industrial wastewaters are potentially hazardous. Gloves and safety glasses should be worn when dispensing these samples.
3.2 Possible exposure to enteric pathogens. Care must be taken to avoid undue exposure.
3.3 A flame is used to sterilize forceps. Maintain the area around the flame clear.
3.4 Contaminated (used) Petri dishes and lab equipment must be placed in Biohazardous waste container. This Biohazardous waste container is autoclaved before disposal.

4. Sample Handling and Preservation

4.1 Samples should be collected in clean, sterile glass or plastic containers.
4.2 If chlorine is in the sample, containers should be treated with 4 drops of 10% Sodium thiosulfate before autoclaving.
4.3 Run test immediately after sampling, or preserve sample at 4°C for a maximum of 6 hours.

5. Reagents

5.1 Peptone powder
5.2 Peptone buffer solution pH 7.00 ± 0.1 (stored at 4°C)
5.3 m-FC media with rosolic acid for fecal coliforms (stored at 4°C)
5.4 Sodium thiosulfate 10% solution
5.5 Lysol disinfectant, 20% solution

6. Equipment & Lab Ware

6.1 Vacuum flask
6.2 Millipore® single use 47 mm Petri dishes with pads
6.3 Millipore® sterilized 47 mm filter
6.4 Forceps
6.5 4.5 X 9 inch sterile sampling bags
6.6 Bunsen burner and striker
6.7 Pipettes and sterile tips
6.8 Sterilized filter holder (plastic or glass)
6.9 Gable topped water bath at 44.5°C ± 0.2°C
6.10 Thermometer
6.11 ASTM Thermometer
6.12 Tower Steam Indicator Strips
6.13 ODO-Clave® Heat Activated Deodorant Pads
6.14 Autoclavable Biohazard waste bags and deposit box
6.15 Autoclave
6.16 Sterile blue sheets
6.17 Indicator tape
6.18 Autoclavable Nalgene® squeeze bottles for peptone

7. Interferences

7.1 Bacteria from the surrounding environment.

7.2 Cross contamination from one sample to the next.

7.3 Lack of aseptic techniques.

8. Procedures

8.1 Steps

1) Clean work area with Lysol disinfectant, 20% solution.

2) Light Bunsen burner with striker.

3) Open sterile filter holder. Use sterile blue sheet as a sterile field. Indicator trip and tape should indicate that the filter has been sterilized.

4) Flame forceps and use it to remove the Indicator Strip without touching anything else except the strip.

5) Use Petri dishes with sterile pad already in dish.

6) Break open ampule of media and pour onto media pad.

7) Decant excess media and cover dish to protect sterile pad.

8) Place the bottom of the sterile filter holder onto the vacuum flask.

9) Flame forceps, remove sterilized filter from packaging and place onto sterilized filter holder (grid side up). Do not touch the filter with anything except the forceps.

10) Place or clamp the top unit onto filter holder.

11) Gently mix sample.

12) In advance, determine sample volume that will yield 20-60 fecal coliform units (FCU).

13) If the volume of sample to be used is 0.1 to 5 mL, pour approximately 10 mL of peptone into filter unit before dispensing sample (Turn on vacuum **after** the sample is introduced).

14) For sample volumes 5 to 50 mL, use sterile pipettes for dispensing into filter unit.

15) Do not touch the inside of the filter holder unit. Do not allow the pipette tip to touch the filter.

16) For sample volumes 50 to 100 mL, pour sample into cylinder and use the scale on the side of the cylinder for measurement.

17) Turn on vacuum.

18) Once sample has filtered through, turn off vacuum.

19) Rinse top of the filter unit with peptone two times using autoclaved peptone in a Nalgene® squeeze bottle.

20) Turn on vacuum to drain peptone.

21) Turn off vacuum.

22) Flame forceps.

23) Remove top of the filter unit and place on the sterile blue field.

24) Open Petri dish.

25) Use sterile (flamed) forceps to grab the edge of the filter and remove it from the filter holder unit.

26) Place filter, grid side up, onto edge of the Petri dish and gently slide it onto the surface of the media saturated pad.

27) Replace Petri dish cover.

28) Place Petri dishes into a 4.5 X 9 inch sterile sampling bag. Make sure not contaminate the inside of the bag.

29) Seal bag.

30) Place bag, with Petri dishes face down, into water bath at 44.5°C ± 0.2°C for 24 ± 2 hours.

31) Log initials, time, and date in the Microbiology bench sheet.

32) After 24 ± 2 hours, count blue colonies (See Section 10).

33) Log results, initials, time, and date in the Microbiology bench sheet.

8.2 Before and After Blanks

1) Blanks must be analyzed before and after filtration of a set of samples.

2) Before any sample is filtered, place a sterile filter in the filter holder unit.

3) Add 50 mL peptone buffer to filter unit.

4) Turn on vacuum and filter the buffer, this will be the Before Blank.

5) Remove and plate filter as indicated in Section 8.1.

6) Proceed with sample filtration and plating.

7) Run a Known Positive after all samples have been filtered (See Section 8.3).

8) Place a sterile filter in the filter holder unit.

9) Add 50 mL peptone buffer to filter unit.

10) Turn on vacuum and filter the buffer, this will be the After Blank.

11) Remove and plate filter as indicated in Section 8.1.

12) Log results, initials, time, and date in the Microbiology bench sheet.

8.3 Known Positive

1) After all samples have been filtered, a Known Positive is filtered and plated to ensure growth.

2) Place a sterile filter in the filter holder unit.

3) Add 10-20 mL peptone buffer to filter unit, then pipet 1.0 mL of mixed liquor (or suitable sample with known fecal coliforms) into filter unit.

4) Turn on vacuum and filter the sample, this will be the Known Positive.

5) Remove and plate filter as indicated in Section 8.1.

6) Log results, initials, time, and date in the Microbiology bench sheet.

8.4 Peptone Buffer Preparation

1) Measure 1.0 g Peptone powder into 1L volumetric flask.

2) Bring to volume with Nanopure water. Mix well.

3) Pour Peptone buffer into Nalgene® autoclavable squeeze bottle.

4) Take a sample of the buffer and measure pH, it should be 7.00 ± 0.1.

5) Loosely screw caps/dispensers onto squeeze bottles.

6) Autoclave Peptone buffer solutions.

7) Allow to cool, then transfer squeeze bottles to Micro fridge.

8.5 Helpful Hints

1) If the bacterial density of the sample is unknown, filter and plate out several volumes or dilutions in order to achieve a countable density. The volumes and/or dilutions should be expected to yield a countable membrane. In addition, select two additional quantities representing one-tenth and ten times this volume, respectively.

2) Separate filter holder units may be required during a set of samples. These will be indicated in the bench sheet.

3) Do not use damaged or bent membrane filters.

4) Rinse the filter unit thoroughly with Peptone buffer to avoid cross contamination.

9. QA/QC Requirements

9.1 Before and After Blanks must be run with each set of samples tested.

9.2 One duplicate per test series must be run.

9.3 One “Known positive” must be run per test series.

10. Expected Results

10.1 KPDES Permit Requirements

200 CFU/100 mL for Monthly geometric mean (GED)
400 CFU/100 mL for Maximum Weekly GED

In the event that the GED is exceeds the KPDES permit requirements, notify the Plant Superintendent and the Laboratory Supervisor.

10.2 Process Ranges

< 1 to >120,000 CFU/100 mL

11. Data Analysis and Calculations

11.1 See the following pages.

12. Bibliography

12.1 U.S. EPA. Microbiological Methods for Monitoring the Environment: Water and Wastes. EPA 600/8-78-017. U.S. Environmental Protection Agency; Environmental Monitoring and Support Laboratory, Office of Research and Development, Washington, DC, 1978. Page 124.

12.2 Kentucky Department for Environmental Protection, Kentucky Division of Water and the Kentucky Division of Compliance Assistance. Discharge Monitoring Report Manual. 2009. August 10, 2009 revision. 28 pp.

FECAL COLIFORM CALCULATIONS

The ways the answers are presented vary with the number of colonies on the plate.

A. Countable plates [1] with 20-60 Blue colonies.

$$\text{Fecal Coliforms/100 mL} = \frac{\text{Number of Blue colonies counted}}{\text{Volume of sample filtered (mL)}} \text{x} 100$$

Example: 40 Blue colonies are counted and 50 mL of sample was used.

$$\text{Fecal Coliforms/100 mL} = \frac{40}{50} \text{x} 100 = 80$$

B. Countable plates with less than 20 Blue colonies.

- If only one plate has been set-up, calculate as shown, but place an approximately equal to (≈) sign [2] in front of the answer.

$$\text{Fecal Coliforms/100 mL} = \frac{18}{50} \text{x} 100 = \approx 36$$

- If more than one plate has been set-up, calculate the total number of colonies from all the plates and the total volume of sample used for all the plates. Use these totals to calculate the number of coliforms per 100 mL.

Example:	10 Blue colonies	50 mL Sample
	5 Blue colonies	40 mL Sample
	3 Blue colonies	10 mL Sample

$$\text{Fecal Coliforms/100 mL} = \frac{10 + 5 + 3}{50 + 40 + 10} \text{x} 100 = \approx 18$$

C. Plates with no Blue colonies.

- Do not use zero in the calculations. Place the number one in the equation and use the largest volume of sample. Report as less than (<).

Example:	**0 Blue colonies**	**50 mL Sample**
	0 Blue colonies	40 mL Sample
	0 Blue colonies	10 mL Sample

D. Countable plates with more than 60 Blue colonies.

- If only one plate has been set-up, calculate as shown, but place a approximately equal to (≈) sign in front of the answer.

$$\text{Fecal Coliforms/100 mL} = \frac{95}{50} \text{x} 100 = \approx 190$$

- If more than one plate has been set-up, use the plate with the highest dilution (the lowest sample volume). Place an approximately equal to (≈) sign in front of the answer.

Example:	150 Blue colonies	50 mL Sample
	100 Blue colonies	40 mL Sample
	80 Blue colonies	**10 mL Sample**

$$\text{Fecal Coliforms/100 mL} = \frac{80}{10} \text{x} 100 = \approx 800$$

E. Uncountable plates with more than 60 Blue colonies (TNTC).

- If a sample contains colonies that are too numerous to count, conduct enough dilutions in these tests to obtain discrete ("real number") values. Reporting a non-numerical value, such as TNTC, is unacceptable because you cannot average non-numerical values.
- For samples in which subsequent dilutions do not produce a discrete value and are too numerous to count, **report results as 60,000**. You would also use this value in the geometric mean calculation. (KDEP DMR Manual, 2009).

F. To calculate the Geometric Mean (G.E.D.).

- Zeros shall be recorded as < 1.

- To calculate the logs of numbers with greater than, less than, and approximately equal to sign, drop the sign. Do the math and then replace the sign as shown below.

- If a column has only numbers without signs and numbers with less than signs, then the total of this column will have a less than sign.

- If a column has numbers without signs, numbers with less than signs, and numbers with greater than sign, then the total of this column will have a less than sign.

- If a column has only numbers without signs and numbers with greater than signs, then the total of this column will have a greater than sign.

- If a column has any numbers with approximately equal to signs, then the total of this column will have an approximately equal to sign.

Example:

Fecal Coliform/100 mL	Logarithm
100	2.00000
50	1.69897
< 1	0.00000
» 10	1.00000
60	1.77815
80	1.90309
150	2.17609
> 60000	4.77815
< 83	15.33445

15.33445 / 8 = 1.91681

1.91681 = 83

[1] Countable plate is a plate that has a number of well formed Blue colonies that can be easily counted (0 to about 150). The EPA states, that a count of 20 to 60 colonies is the desired range for Fecal Coliform plates.

[2] Enter these signs as follows: The Font is Symbol. The size is 11.

Nomenclature	Symbols	Keystrokes
Approximately equal to	»	Hold down the Alt-Key and type 0187
Greater Than	>	Hold down the Shift-Key and type >
Less Than	<	Hold down the Shift-Key and type <
Greater Than or equal to	≥	Hold down the Alt-Key and type 0179
Less Than or equal to	≤	Hold down the Alt-Key and type 0163

Note: Per KDEP letter dated March 17, 2006. When entering data in the DMR form, if there is a greater than sign (>) in the results, enter the number without the sign. A note must be made that the number had a greater than sign and as to why there was a greater than sign. No changes are needed when other signs are used. This is for the monthly and weekly average data.

m-ColiBlue24 Method for the Determination of Total Coliforms and *E. coli*
HACH Method 10029
Federal Register (Dec. 1, 1999, FR Vol. 64, No. 230, 67449-67467)
40 CFR 141

1. Scope, Significance to Process and Application

1.1 The coliform analysis is applicable to investigations of stream pollution, raw water sources, and wastewater treatment systems.

1.2 m-ColiBlue24 broth simultaneously detects and identifies both total coliforms and *Escherichia coli* (*E. coli*).

2. Summary of Method

2.1 Executive Summary

The sample is filtered through a 0.45 µm Millipore® membrane filter. The filter is placed on a Pall® filter pad containing media in a sterile Petri dish. The samples are then incubated at 35.0°C ± 0.5°C for 24 hours ± 2 hours. Colonies are counted, blue colonies are enumerated as *E. coli* and other coliforms are red; total coliforms are the sum of the two.

2.2 Discussion

m-ColiBlue24 is a single-step MF procedure that incorporates specific non-coliform growth inhibitors and a selective enzymatic indicator to allow for simultaneous detection and quantitation of both *E. coli* and total coliforms. Fecal coliforms are defined as. The major species of gram-negative, non-spore forming rods is *Escherichia coli*, which indicates fecal pollution and the presence of enteric pathogens. m-ColiBlue24 method has been approved by the U.S. EPA for monitoring drinking water and wastewater using a 24-hour incubation period. It can also be used to detect coliforms in other types of water (i.e. surface, ground, well, recreational).

3. Health & Safety Precautions

3.1 All municipal and industrial wastewaters are potentially hazardous. Gloves and safety glasses should be worn when dispensing these samples.
3.2 Possible exposure to enteric pathogens. Care must be taken to avoid undue exposure.
3.3 A flame is used to sterilize forceps. Maintain the area around the flame clear.
3.4 Contaminated (used) Petri dishes and lab equipment must be placed in Biohazardous waste container. This Biohazardous waste container is autoclaved before disposal.

4. Sample Handling and Preservation

4.1 Samples should be collected in clean, sterile glass or plastic containers.
4.2 If chlorine is in the sample, containers should be treated with 4 drops of 10% Sodium thiosulfate before autoclaving.
4.3 Run test immediately after sampling, or preserve sample at 4°C for a maximum of 6 hours.

5. Reagents

5.1 Peptone powder
5.2 Peptone buffer solution pH 7.00 ± 0.1 (stored at 4°C)
5.3 m-ColiBlue24 media in plastic ampule (stored at 4°C)
5.4 Sodium thiosulfate 10% solution
5.5 Lysol disinfectant, 20% solution

6. Equipment & Lab Ware

6.1 Vacuum flask
6.2 Pall® Life Sciences single use 50 mm Petri dishes with pads
6.3 Millipore® sterile 0.45 µm 47 mm filter
6.4 Forceps
6.5 4.5 X 9 inch sterile sampling bags
6.6 Bunsen burner and striker
6.7 Pipettes and sterile tips
6.8 Sterilized filter holder (plastic or glass)
6.9 Gable topped water bath at 35.0°C ± 0.5°C
6.10 Thermometer
6.11 ASTM Thermometer
6.12 Tower Steam Indicator Strips
6.13 ODO-Clave® Heat Activated Deodorant Pads
6.14 Autoclavable Biohazard waste bags and deposit box
6.15 Autoclave
6.16 Sterile blue sheets
6.17 Indicator tape
6.18 Autoclavable Nalgene® squeeze bottles for peptone

7. Interferences

7.1 Bacteria from the surrounding environment.
7.2 Cross contamination from one sample to the next.
7.3 Lack of aseptic techniques.

8. Procedures

8.1 Steps

1) Clean work area with Lysol disinfectant, 20% solution.

2) Light Bunsen burner with striker.

3) Open sterile filter holder. Use sterile blue sheet as a sterile field. Indicator trip and tape should indicate that the filter has been sterilized.

4) Use **Pall®** petri dishes with sterile pad already in dish.

5) Break open m-ColiBlue ampule of media and pour the contents evenly over the absorbent pad and place the lid on the Petri dish.

6) Decant excess media and cover dish to protect sterile pad.

7) Place the bottom of the sterile filter holder onto the vacuum flask.

8) Flame forceps, remove sterilized filter from packaging and place onto sterilized filter holder (grid side up). Do not touch the filter with anything except the forceps.

9) Place or clamp the top unit onto filter holder.

10) Gently mix sample.

11) In advance, determine sample volume that will yield 20-60 fecal coliform units (FCU).

12) If the volume of sample to be used is 0.1 to 5 mL, pour approximately 10 mL of peptone into filter unit before dispensing sample (Turn on vacuum **after** the sample is introduced).

13) For sample volumes 5 to 50 mL, use sterile pipettes for dispensing into filter unit.

14) Do not touch the inside of the filter holder unit. Do not allow the pipette tip to touch the filter.

15) For sample volumes 50 to 100 mL, pour sample into cylinder and use the scale on the side of the cylinder for measurement.

16) Turn on vacuum. Once sample has filtered through, turn off vacuum.

17) Rinse top of the filter unit with peptone two times using autoclaved peptone in a Nalgene® squeeze bottle.

18) Turn on vacuum to drain peptone. Turn off vacuum.

19) Flame forceps.

20) Remove top of the filter unit and place on the sterile blue field.

21) Open Petri dish.

22) Use sterile (flamed) forceps to grab the edge of the filter and remove it from the filter holder unit.

23) Place filter, grid side up, onto edge of the Petri dish and gently slide it onto the surface of the media saturated pad. Check for trapped air under the filter and make sure the filter touches the entire pad.

24) Replace the Petri dish lid.

25) Place Petri dishes into a 4.5 X 9 inch sterile sampling bag. Make sure not contaminate the inside of the bag. Seal bag.

26) Place bag, with Petri dishes face down, into water bath at 35.0°C ± 0.5°C for 24 ± 2 hours.

27) Log initials, time, and date in the Microbiology bench sheet.

28) After 24 ± 2 hours, count the colonies. Use a 10 to 15X stereoscopic microscope, if necessary. If no red or blue colonies appear, the sample can be considered negative. If red or blue colonies appear at 24 hours incubation, the result is positive for total coliforms or *E. coli*, respectively. No confirmation step is required.

29) Log results, initials, time, and date in the Microbiology bench sheet.

8.2 Before and After Blanks

1) Blanks must be analyzed before and after filtration of a set of samples.
2) Before any sample is filtered, place a sterile filter in the filter holder unit.
3) Add 50 mL peptone buffer to filter unit.
4) Turn on vacuum and filter the buffer, this will be the Before Blank.
5) Remove and plate filter as indicated in Section 8.1.
6) Proceed with sample filtration and plating.
7) Place a sterile filter in the filter holder unit.
8) Add 50 mL peptone buffer to filter unit.
9) Turn on vacuum and filter the buffer, this will be the After Blank.
10) Remove and plate filter as indicated in Section 8.1.
11) Log results, initials, time, and date in the Microbiology bench sheet.

8.3 Peptone Buffer Preparation

See SOP-Fecal Coliform, Section 8.4 for peptone buffer preparation.

8.4 Helpful Hints

1) If the bacterial density of the sample is unknown, filter and plate out several volumes or dilutions in order to achieve a countable density. The volumes and/or dilutions should be expected to yield a countable membrane. In addition, select two additional quantities representing one-tenth and ten times this volume, respectively.

2) Separate filter holder units may be required during a set of samples. These will be indicated in the bench sheet.

3) Do not use damaged or bent membrane filters.

4) Rinse the filter unit thoroughly with Peptone buffer to avoid cross contamination.

9. QA/QC Requirements

9.1 Before and After Blanks must be run with each set of samples tested.
9.2 One duplicate per test series must be run.

10. Expected Results

10.1 KPDES Permit Requirements
Currently there are no permit requirement on *E.coli*.

11. Data Analysis and Calculations

11.1 See SOP – Fecal Coliform.
11.2 Blue colonies are counted as *E. coli* and red colonies are other coliforms; total coliforms are the sum of the two.

12. Bibliography

12.1 HACH Method 10029, Coliforms: Membrane Filtration. 2009.

12.2 U.S. EPA. Microbiological Methods for Monitoring the Environment: Water and Wastes. EPA 600/8-78-017. U.S. Environmental Protection Agency; Environmental Monitoring and Support Laboratory, Office of Research and Development, Washington, DC, 1978. Page 124.

12.3 Federal Register. Dec. 1, 1999, FR Vol. 64, No. 230, 67449-67467.

12.4 Code of Federal Regulations (CFR). 40 CFR 141. National primary drinking water regulations. U.S. Environmental Protection Agency.

Hardness, Total (mg/L as $CaCO_3$; Titrimetric, EDTA)
HACH Method 8213 Hardness, Total with a Digital Titrator
Standard Methods 2340-C, U.S. EPA Method 130.2

1. Scope, Significance to Process and Application

1.1 Hardness is defined as a characteristic of water which represents the total concentration of calcium and magnesium expressed as their calcium carbonate equivalent.

2. Summary of Method

2.1 Executive Summary
The EDTA titration method measures the calcium and magnesium ions and may be applied with appropriate modification to any kind of water. The procedure affords a means of rapid analysis.

2.2 Discussion
Sample is titrated with 0.800 M EDTA titrant until the color changes from red to pure blue. The EDTA complexes calcium and magnesium ions. Color change occurs when all calcium and magnesium ions are complexed, indicating the end point of titration.

3. Health & Safety Precautions

3.1 Watch out for broken glass from cylinders and porcelain dishes.
3.2 Wastewater samples have the potential to be hazardous, use appropriate caution.
3.3 Use of Nitric Acid, use appropriate caution.

4. Sample Handling and Preservation

4.1 Collect samples in plastic or glass containers that have been washed with a detergent and rinsed with tap water, 1:1 Nitric Acid Solution, and Nanopure water.
4.2 Analyze immediately or perform preservation.
4.3 To preserve the sample, add 1.5 mL of Nitric Acid per liter of sample. Check the sample to assure that the pH is 2 or less.
4.4 Store samples at 4^oC or below.

5. Reagents

5.1 Hardness 1 Buffer Solution.
5.2 ManVer 2 Hardness Indicator Powder Pillow.
5.3 0.800 M EDTA Titration Cartridge.

6. Equipment & Lab Ware

6.1 HACH Digital burette.
6.2 100 mL graduated cylinder.
6.3 Porcelain dish.
6.4 Stirrer and magnet.

7. Interferences

7.1 Some metal ions interfere by causing fading or indistinct endpoint or by stoichiometric consumption of EDTA.
7.2 Suspended or colloidal organic matter also may interfere with the endpoint.

8. Procedures

8.1 Steps

1) Attach a clean, horse-shoe bent delivery tube to a 0.800 M EDTA Titration Cartridge connected to the HACH digital burette.

2) Flush the delivery tube by turning the delivery knob on the burette to eject a few drops of titrant. Reset the counter to zero and wipe tip.

3) Measure 100 mL of sample with a clean 100-mL graduated cylinder.

4) Pour the sample into a clean porcelain dish.

5) Add 2 mL of Buffer Solution, Hardness 1, and stir.

6) Add the contents of one ManVer 2 Hardness Indicator Powder Pillow and stir.

7) Titrate the sample with 0.800 M EDTA titrant until the color changes from red to pure blue.

8) Read the concentration of total hardness (as mg/L $CaCO_3$) directly from the digital counter window.

9. QA/QC Requirements

9.1 None required.

10. Expected Results

10.1 KPDES Permit Requirements
None required. Monitored quarterly by 24-hour composite sample, concurrent with whole effluent testing.

10.2 Process Ranges [1]
Average Annual Hardness = 247 mg/L
Minimum monthly average = 177 mg/L
Maximum monthly average = 321 mg/L

[1] Based on DMR data (January 1, 2001 through June 30, 2006).

11. Data Analysis and Calculations

11.1 None required.

12. Bibliography

12.1 HACH Water Analysis Handbook. Method 8213 Hardness, Total with a Digital Titrator. 2nd Edition. 1992. HACH Company, Loveland, CO.

12.2 Standard Methods 2340-C. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

12.3 U.S. EPA. Method 130.2. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

pH (Electrometric)
EPA Method 150.1 pH. Issued 1971 (Editorial revision 1978 and 1982)

1. Scope, Significance to Process and Application

1.1 At a given temperature the intensity of the acidic or basic character of a solution is indicated by a pH or hydrogen ion activity.
1.2 A pH meter is accurate and reproducible to 0.1 pH unit with a range of 0 to 14 and equipped with a temperature compensation adjustment.

2. Summary of Method

2.1 Executive Summary
The pH of a sample is determined electrometrically using either a glass electrode in combination with a reference potential or a combination electrode.

2.2 Discussion
There is no color change during analysis. pH stands for power of hydrogen, a measure of hydrogen ion concentration in solution.

3. Health & Safety Precautions

3.1 Watch out for broken glass from beakers and cylinders.
3.2 Wastewater samples should be considered potentially hazardous. Use proper personal protective equipment.

4. Sample Handling and Preservation

4.1 Samples should be analyzed as soon as possible within a 15 minute time window.
4.2 Sample containers should be filled completely and kept sealed prior to analysis.

5. Reagents

5.1 Buffer Solution pH – 4.00 (color coded red)
5.2 Buffer Solution pH – 7.00 (color coded yellow)
5.3 Buffer Solution pH – 10.00 (color coded blue)
5.4 Buffer Solution pH – 6.86
5.5 Nanopure Grade Laboratory Water
5.6 Reference Electrode Filling Solution

6. Equipment & Lab Ware

6.1 Fisher Scientific AR50 pH Meter.
6.2 Stirrer probe.

7. Interferences

7.1 pH measurements are affected by temperature and can cause the reading to drift.
7.2 Grease and other debris on the probe can cause inaccurate readings.

8. Procedures

8.1 Calibration

1) Before calibrating, ensure the hole on the side of the pH probe is open.

2) Weekly - Replace Reference Filling Solution.
   a. Push down on probe to release filling solution.
   b. Insert Reference Filling Solution bottle tip into hole of probe and flush with solution.
   c. Refill probe with Reference Electrode Filling Solution until inner workings of probe are covered.

3) Touch the meter screen (anywhere), until you hear a beep.

4) Touch the "*pH* button" and wait until you hear a beep.

5) Touch the "std button" on the upper right corner of the screen.

6) Place probes into a beaker of Buffer Solution pH – 7.00 (yellow color coded). Turn on stirrer.

7) Touch the "*clear* button" to remove previous standardization.

8) Write down the temperature of the buffer and determine what the pH for the buffer will be at this temperature from chart.

Temperature ^{o}C	Buffer Solution pH – 7.00 (yellow color coded)
10	7.07
15	7.05
20	7.03
25	7.00
30	6.99

9) Type the value for the pH 7.0 buffer, at the given temperature, then press "enter" key.

10) Turn off stirrer and rinse probes with Nanopure water.

11) Place probes into a beaker of Buffer Solution pH – 4.00 (red color coded). Turn on stirrer.

12) Write down the temperature of the buffer and check what the pH for the buffer will be at this temperature from chart.

Temperature ^{o}C	Buffer Solution pH – 4.00 (red color coded)
10	4.00
15	3.99
20	4.00
25	4.00
30	4.00

13) Touch the "*std* button" on the upper right corner of the screen.

14) Type the value for the pH 4.0 buffer, at the given temperature, then press the "enter" key.

15) Turn off stirrer and rinse probes with Nanopure water.

16) Place probes into a beaker of Buffer Solution pH – 10.00 (blue color coded). Turn on stirrer.

17) Write down the temperature of the buffer and determine what the pH for the buffer will be at this temperature from chart.

Temperature ^{o}C	Buffer Solution pH – 10.00 (blue color coded)
10	10.19
15	10.12
20	10.06
25	10.00
30	9.94

18) Touch the "std button" on the upper right corner of the screen.

19) Type the value for the pH 10 buffer, at the given temperature, then press the "enter" key.

20) Turn off stirrer and rinse probes with Nanopure water.

21) Touch the "*meas*" button on the upper right side of the screen. Meter is now calibrated and ready for sample measurements.

22) Record the slope, temperature, buffer values, time calibrated and initials in the bench sheet and calibration folder.

8.2 Sample Measurements

1) Touch the "*meas*" button on the upper right side of the screen.

2) When testing samples for pH, place probe into sample container and record the reading once the measurement has become stable. ("STABLE" will appear once the meter recognizes that the measurement is stable).

3) Turn off stirrer and rinse probes with Nanopure water between samples.

4) When all analytes have been tested for that day, touch the "*mode* button" to put meter into power save mode.

5) Replace plastic sleeve or cap to cover hole in pH probe for safe storage.

6) Store pH probe in pH 7.0 buffer solution.

8.3 Weekend Check Standard

1) For Sunday operators, calibrate meter as described in Section 8.1.

2) Measure pH of Buffer Solution pH – 6.86 to ensure meter is working correctly. Record value of Check Standard in bench sheet.

3) Proceed to measure samples as described in Section 8.2.

8.4 Helpful Hints

1) Avoid strong acids and greasy samples.

2) Make sure that probe is thoroughly rinsed between samples to avoid cross-contamination.

9. QA/QC Requirements

9.1 If sample is not collected properly or analyzed within 15 minutes, another sample must be obtained and analyzed.

9.2 A Check Standard (pH 6.86) must be analyzed on Sunday.

10. Expected Results

10.1 KPDES Plant Effluent Permit Requirements

1) 6.0 mg/L is the lowest pH allowed.
2) 9.0 mg/L is the highest pH allowed.

In the event that analysis results indicate values greater then KPDES permit requirements, retest. If the value indicated by the retest is greater than KPDES permit requirements, **Immediately** notify the Plant Superintendent and the Laboratory Supervisor.

10.2 Process Ranges

Typical values for each plant are:

Town Branch Influent	7.3 – 7.4
Town Branch Effluent	6.7 – 8.9
West Hickman Influent	7.1 – 7.4
West Hickman Effluent	6.4 – 7.7
Blue Sky Influent	7.1 – 7.5
Blue Sky Effluent	6.0 – 7.3

11. Data Analysis and Calculations

11.1 None required.

12. Bibliography

12.1 U.S. EPA. Method 150.1 pH (Electrometric) Issued 1971 (Editorial revision 1978 and 1982). Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020; U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

12.2 AR50 Fisher Scientific User Manual. Fisher Scientific

12.3 Code of Federal Regulations (CFR). 2003. Guidelines Establishing Test Procedures for the Analysis of Pollutants. 40 CFR 136.3, Title 40, Chapter 1. U.S. Environmental Protection Agency; U.S. Environmental Protection Agency. pg 5-337.

TEMPERATURE to pH CHART

This chart is for color-coded buffers only

Temperature of the Buffer, ºC	pH Buffer 4.00	pH Buffer 7.00	pH Buffer 10.00
0	4.01	7.12	10.34
5	3.99	7.11	10.26
10	4.00	7.07	10.19
15	3.99	7.05	10.12
20	**4.00**	**7.03**	**10.06**
25	4.00	7.00	10.00
30	4.01	6.99	9.94
35	4.02	6.99	9.90
40	4.03	6.97	9.85
50	4.06	6.97	9.78
60	4.09	6.98	9.70

Total Phosphorus Analysis
Ascorbic Acid Method
Phosphorus, Reactive (Orthophosphate), HACH Method 10209
Total Phosphorus, HACH Method 10210
TNT+ 843 LR (0.15 to 4.50 mg/L PO_4^{3-} or 0.05 to 1.50 mg/L PO_4–P)
TNT+ 844 HR (1.5 to 15.0 mg/L PO_4^{3-} or 0.5 to 5.0 mg/L PO_4–P)
TNT+ 845 UHR (6 to 60 mg/L PO_4^{3-} or 2 to 20 mg/L PO_4–P)
Reactive Phosphorus, EPA Method 365.1
Total Phosphorus, EPA Method 365.3

1. Scope, Significance to Process and Application

1.1 Phosphorus in wastewater is almost always present in the form of phosphates. There are three major classes of phosphates; (1) Orthophosphates (fertilizer is major source), (2) Polyphosphates (detergents and cleaning agents are major sources), and (3) Organic Phosphates (biological waste is major source). Organic Phosphates are also formed from orthophosphates during biological treatment of waste streams. Analysis of Total Phosphorus includes all of the aforementioned forms of Phosphorus. The reduction of Total Phosphorus levels throughout the wastewater treatment process is highly important, as phosphorus concentrations in plant effluents must be low enough (See Sec.10.1, Permit Requirements) to avoid detrimental effects on the receiving environment, such as algae blooms.

2. Summary of Method

2.1 Executive Summary

Total phosphorus analysis at Town Branch Laboratory refers to the spectrophotometric analysis of all phosphorus forms in a water/wastewater sample that has been digested.

2.2 Discussion

Phosphates present in organic and condensed inorganic forms (meta-, pyro- or other polyphosphates) are first converted to reactive orthophosphate in the Total Phosphorus procedure. Treatment of the sample with acid and heat provides the conditions for hydrolysis of the condensed inorganic forms. Organic phosphates are also converted to orthophosphates in the Total Phosphorus procedure by heating with acid and persulfate. The Reactive Phosphorus procedure measures only the reactive (ortho) phosphorus present in the sample. The reactive or orthophosphate ions react with molybdate and antimony ions in an acidic solution to form an antimonyl phosphomolybdate complex, which is reduced by ascorbic acid to phosphomolybdenum blue. Test results are measured at 890 nm with a HACH DR5000 spectrophotometer.

3. Health & Safety Precautions

3.1 During sample digestion, the digester block is HOT (100˚C), Burn Hazard. Use digester block safety shields. In addition, the capped glass sample vials contain hot (100˚C) acidic solution under pressure.
3.2 Watch out for broken glass from beakers and cylinders.
3.3 Wastewater samples should be considered potentially hazardous. Use proper personal protective equipment.

4. Sample Handling and Preservation

4.1 Collect samples in plastic or glass bottles that have been acid cleaned with 1:1 Hydrochloric acid solution and rinsed with Nanopure water.
4.2 Do not use commercial detergents containing phosphate for cleaning glassware used in this test.
4.3 Analyze samples immediately after collection for best results.
4.4 If prompt analysis is impossible, preserve samples for Total Phosphorus up to 28 days by adjusting the pH to 2 or less with concentrated Sulfuric acid (about 2 mL per liter) and storing at 4 °C.
4.5 Samples to be analyzed for Reactive Phosphorus should not be preserved with acid: store Reactive Phosphorus samples at 4 °C and analyze within 48 hours.
4.6 Warm stored samples to 15–25 °C and neutralize with 5.0 N NaOH before analysis if acid has been added.

5. Reagents

5.1 Phosphorus, Reactive and Total, TNTplus LR Reagent Set (HACH TNT843)
5.2 Phosphorus, Reactive and Total, TNTplus HR Reagent Set (HACH TNT844)
5.3 Phosphorus, Reactive and Total, TNTplus UHR Reagent Set (HACH TNT845)
5.4 Nanopure Water
5.5 Phosphate Standard Solution 100 mg/L as PO_4^{3-}

6. Equipment & Lab Ware

6.1 HACH DRB200 Reactor with Test'N Tube block and safety shields
6.2 TNTplus reactor adapter sleeves (16-mm to 13-mm diameter)
6.3 HACH DR5000 Spectrophotometer
6.4 Adjustable volume pipettes (100-1000 µL) with tips
6.5 Adjustable volume pipettes (1000-5000 µL) with tips
6.6 Test Tube Rack
6.7 Vials with 9 and 19 mL of Nanopure water for dilutions

7. Interferences

7.1 Do not use commercial detergents containing phosphate for cleaning any lab ware utilized in this method.
7.2 Excess Turbidity.

8. Procedures

8.1 TNTplus 843 Total Phosphorous LR (0.05–1.50 mg/L PO_4–P)

1) Turn on the DRB200 Reactor. Heat to 100 °C. For DRB200 Reactors with 16-mm wells, make sure the 16- to 13-mm adapter sleeves are in each well before turning on the reactor.
2) Carefully remove the protective foil lid from the DosiCap™ *Zip*. Unscrew the cap from the vial.
3) Carefully pipet **2.0 mL** of sample into the vial.
4) Flip the DosiCap *Zip* over so the reagent side faces the vial. Screw the cap tightly onto the vial.
5) Shake the capped vial with 2–3 times to dissolve the reagent in the cap. Verify that the reagent has dissolved by looking down through the open end of the DosiCap *Zip*.
6) Insert the vial in the DRB200 Reactor. Close the protective cover. Heat for 1 hour at 100 °C.
7) After the timer expires, carefully remove the hot vial from the reactor. Insert it in a test tube rack and allow to cool to room temperature (15–25 °C).
8) Pipet 0.2 mL (200 µL) of Reagent B into the cooled vial. Immediately close the Reagent B container.
9) Screw a grey DosiCap C onto the vial.
10) Invert the capped vial 2–3 times to dissolve the reagent in the DosiCap.
11) Wait 10 minutes.
12) When the timer expires, invert the vial again 2–3 times. Clean the vial with a Kim-Wipe and insert it into the DR5000 cell holder. The instrument reads the barcode, then selects and performs the correct test. Results are in mg/L PO_4. No instrument Zero is required.
13) Note and record the indicated values on the bench sheet

8.2 TNTplus 843 Reactive Phosphorous LR (0.15–4.50 mg/L PO_4^{3-})

1) Carefully pipet **2.0 mL** of sample into the vial.
2) Pipet 0.2 mL (200 µL) of Reagent B into the vial. Immediately close the Reagent B container.
3) Screw a grey DosiCap C onto the vial.
4) Invert the capped vial 2–3 times to dissolve the reagent in the DosiCap.

5) Wait 10 minutes.
6) When the timer expires, invert the vial again 2–3 times.
7) Clean the outside of the vial with a Kim-Wipe and insert it into the DR5000 cell holder. The instrument reads the barcode, then selects and performs the correct test. Results are in mg/L PO_4. No instrument Zero is required.
8) Note and record the indicated values on the bench sheet

8.3 TNTplus 844 Total Phosphorous LR HR (0.5-5.0 mg/L PO_4–P)

1) Turn on the DRB200 Reactor. Heat to 100 °C. For DRB200 Reactors with 16-mm wells, make sure the 16- to 13-mm adapter sleeve are in each well before turning on the reactor.
2) Carefully remove the protective foil lid from the DosiCap™ *Zip*. Unscrew the cap from the vial.
3) Carefully pipet **0.5 mL (500 µL)** of sample into the vial.
4) Flip the DosiCap *Zip* over so the reagent side faces the vial. Screw the cap tightly onto the vial.
5) Shake the capped vial 2–3 times to dissolve the reagent in the cap. Verify that the reagent has dissolved by looking down through the open end of the DosiCap *Zip*.
6) Insert the vial in the DRB200 Reactor. Close the protective cover. Heat for 1 hour at 100 °C.
7) After the timer expires, carefully remove the hot vial from the reactor. Insert it in a test tube rack and allow to cool to room temperature (15–25 °C).
8) Pipet 0.2 mL (200 µL) of Reagent B into the cooled vial. Immediately close the Reagent B container.
9) Screw a grey DosiCap C onto the vial.
10) Invert the capped vial 2–3 times to dissolve the reagent in the DosiCap.
11) Wait 10 minutes.
12) When the timer expires, invert the vial again 2–3 times.
13) Clean the outside of the vial with a Kim-Wipe and insert it into the DR5000 cell holder. The instrument reads the barcode, then selects and performs the correct test. Results are in mg/L PO_4. No instrument Zero is required.
14) Note and record the indicated values on the bench sheet

8.4 TNTplus 844 Reactive Phosphorous HR (1.5-15.0 mg/L PO_4^{3-})

1) Carefully pipet **0.5 mL** of sample into the vial.
2) Pipet 0.2 mL (200 µL) of Reagent B into the vial. Immediately close the Reagent B container.
3) Screw a grey DosiCap C onto the vial.
4) Invert the capped vial 2–3 times to dissolve the reagent in the DosiCap. Install the Light Shield if applicable.

5) When the timer expires, invert the vial again 2–3 times.
6) Clean the outside of the vial with a Kim-Wipe and insert it into the DR5000 cell holder. The instrument reads the barcode, then selects and performs the correct test. Results are in mg/L PO_4. No instrument Zero is required.
7) Note and record the indicated values on the bench sheet

8.5 TNTplus 845 Total Phosphorous LR UHR (2-20 mg/L PO_4–P)

1) Turn on the DRB200 Reactor. Heat to 100 °C. For DRB200 Reactors with 16-mm wells, make sure the 16- to 13-mm adapter sleeve are in each well before turning on the reactor.
2) Carefully remove the protective foil lid from the DosiCap™ *Zip*. Unscrew the cap from the vial.
3) Carefully pipet **0.4 mL (400 µL)** of sample into the vial.
4) Flip the DosiCap ***Zip*** over so the reagent side faces the vial. Screw the cap tightly onto the vial.
5) Shake the capped vial 2–3 times to dissolve the reagent in the cap. Verify that the reagent has dissolved by looking down through the open end of the DosiCap *Zip*.
6) Insert the vial in the DRB200 Reactor. Close the protective cover. Heat for 1 hour at 100 °C.
7) After the timer expires, carefully remove the hot vial from the reactor. Insert it in a test tube rack and allow to cool to room temperature (15–25 °C).
8) Pipet 0.5 mL (500 µL) of Reagent B into the cooled vial. Immediately close the Reagent B container.
9) Screw a grey DosiCap C onto the vial.
10) Invert the capped vial 2–3 times to dissolve the reagent in the DosiCap.
11) Wait 10 minutes.
12) When the timer expires, invert the vial again 2–3 times.
13) Clean the outside of the vial with a Kim-Wipe and insert it into the DR5000 cell holder. The instrument reads the barcode, then selects and performs the correct test. Results are in mg/L PO_4. No instrument Zero is required.
14) Note and record the indicated values on the bench sheet

8.6 TNTplus 845 Reactive Phosphorous UHR (6-60 mg/L PO_4^{3-})

1) Carefully pipet **0.4 mL** of sample into the vial.
2) Pipet 0.5 mL (500 µL) of Reagent B into the vial. Immediately close the Reagent B container.
3) Screw a grey DosiCap C onto the vial.
4) Invert the capped vial 2–3 times to dissolve the reagent in the DosiCap.
5) Wait 10 minutes.
6) When the timer expires, invert the vial again 2–3 times.

7) Clean the outside of the vial with a Kim-Wipe and insert it into the DR5000 cell holder. The instrument reads the barcode, then selects and performs the correct test. Results are in mg/L PO_4. No instrument Zero is required.
8) Note and record the indicated values on the bench sheet

8.7 **Reagent Blanks**

A reagent blank can be measured and the value subtracted from the results of each test performed using the same reagent lot number. Use Nanopure water in place of sample and perform the Total Phosphorus, Method 10210 or the Reactive Phosphorus, Method 10209 test.

To subtract the value of the blank from a series of measurements:
1. Measure the blank as in step 12 of the Total Phosphorus, Method 10210 test or step 7 of the Reactive Phosphorus, Method 10209 test.
2. Activate the Reagent Blank feature. The measured value of the blank is shown in the highlighted box.
3. Accept the value shown. The reagent blank value will be subtracted from all results until the function is turned off or a different method is selected.

Alternately, the blank can be recorded and entered at any later time by pressing the highlighted box and using the keypad to enter the value.

8.8 **Sample Blanks**

Color or turbidity in samples can cause high results. The digestion in the total phosphate procedure usually destroys all color and turbidity and a sample blank is not required.

To compensate for color or turbidity in the reactive phosphate procedure, the color forming reagent that is present in the DosiCap C is not added.

To determine the sample blank for reactive phosphorus:
1. Run the Reactive Phosphorus, Method 10209 test, but do not add the DosiCap C in step 3.
2. Cap the vial with the original DosiCap *Zip* but do not remove the foil. Use the side of the cap without the reagent.
3. Subtract the value obtained in step 7 from the value obtained on the original reactive phosphate sample to give the corrected sample concentration.

Alternatively, reactive phosphate samples that contain only turbidity may be first filtered through a membrane filter and then analyzed. Samples without color or turbidity do not require sample blanks.

8.9 **Helpful Hints**

1) Analysis results are directly proportional to sample volumes; therefore it is very important that accurate sample volume measurements are made. Correct the test results for volume dilutions.

2) The TNTplus test vials are cuvettes to be analyzed spectrophotometrically, and must provide a clear optical path. Prior to reading, clean the vials by wiping with a Kim-Wipe.

3) When washing lab ware involved with this method, use only phosphate free cleaning agents, 1:1 HCL solution is recommended, followed by thorough Nanopure water rinse. Note: Most of the lab ware used in this method is disposable.

9. Standard Preparation

A 100 mg/L as PO_4^{3-} Phosphate Standard Solution is used as the stock to make standard dilutions. The stock is located in the Room Temp Fridge.

ULR Low Standard (1.01 mg/L)
Combine 1.00 mL of the 10.1 mg/L High Standard with 9.0 mL Nanopure water.

HR High Standard (10.1 mg/L)
Phosphate Standard solution (100 mg/L) to 200 mL volumetric flask. Dilute to 200 mL with Nanopure water.

10. QA/QC Requirements

10.1 A High Standard (10.1 mg/L) and a Low Standard (1.01 mg/L) must be run with every analytical run.

10.2 A total of 5% of all samples must be run in duplicate.

10.3 Data acceptance criteria:
10.3.1 Analysis values for Standards must agree within 10% of the Standard's known value.
10.3.2 Duplicate values must agree within 5%.

If these criteria are not met, corrective action is indicated. See Quality Assurance Program (QAP) Sec. 15 "Corrective Action Policies and Procedures".

11. Expected Results

11.1 KPDES Permit Requirements

1) Town Branch Wastewater Treatment Plant has no effluent limitations on Total Phosphorus. Plant effluent composite samples are analyzed daily. Monthly and weekly averages are reported.

2) West Hickman Wastewater Treatment Plant effluent limitations are:
   a. November 1st through April 30th - a monthly average of 1 mg/L and a weekly average of 2 mg/L.
   b. May 1st through October 31st - there are no effluent limitations on Total Phosphorus.

   Plant effluent composite samples are analyzed daily. Monthly and weekly averages are reported.

In the event that analysis results indicate values greater than the KPDES permit requirements, retest. If the value indicated by the retest is greater than KPDES permit requirements, **Immediately** notify the Plant Superintendent and the Laboratory Supervisor.

11.2 Process Ranges

Typical phosphorus concentration values for plant influent vary primarily due to rainfall. Within the treatment process phosphorus concentrations can vary due to microbiological processes involving the release and uptake of phosphorus forms. Typical values for each plant are:

Town Branch Influent	4 mg/L to 9 mg/L
Town Branch Effluent	2 mg/L to 4 mg/L
West Hickman Influent	2 mg/L to 12 mg/L
West Hickman Effluent	<1.0 mg/L

12. Data Analysis and Calculations

12.1 Concentration values are read directly from the DR5000 spectrophotometer.

13. Bibliography

12.1 HACH. DOC316.53.01124. Phosphorus, Reactive (Orthophosphate) and Total Phosphorus. TNTplus 843. HACH Company, Loveland, CO, 2008.

12.2 HACH. DOC316.53.01125. Phosphorus, Reactive (Orthophosphate) and Total Phosphorus. TNTplus 844. HACH Company, Loveland, CO, 2008.

12.3 HACH. DOC316.53.01126. Phosphorus, Reactive (Orthophosphate) and Total Phosphorus. TNTplus 845. HACH Company, Loveland, CO, 2008.

12.4 HACH DR5000 Procedure Manual. HACH Company, Loveland, CO, 2008.

12.5 Standard Methods Part 4500-P. Phosphorous. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

12.6 U.S. EPA Method 365.1 Phosphorous, All Forms (Colorimetric, Automated, Ascorbic Acid). Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

12.7 U.S. EPA Method 365.3 Phosphorous, All Forms (Colorimetric, Ascorbic Acid, Two Reagent). Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

Settleable Solids
EPA Method 160.5 Residue Settleable Matter
Standard Methods Part 2540-F

1. Scope, Significance to Process and Application

1.1 This method is applicable to surface and saline waters, domestic and industrial wastes.
1.2 The practical lower limit of the determination is about 1 mL/L/hour.

2. Summary of Method

2.1 Executive Summary
Settleable solids is the term used for material settling out of suspension within a defined period of time. It may include floating material, depending on the technique. Solids analyses are important in the control of biological and physical wastewater treatment processes and for assessing compliance with regulatory agency effluent limitations. The settled volume of a biological suspension is useful for routine activated sludge plant control.

2.2 Discussion
Settleable matter is measured volumetrically with an Imhoff Cone and a Settlometer after 1 hour. A 1000 mL sample of mixed liquor is allowed to settle and readings are taken after 30 minutes and at 1 hour intervals thereafter.

3. Health & Safety Precautions

3.1 Wastewater samples should be considered potentially hazardous. Use proper personal protective equipment.

4. Sample Handling and Preservation

4.1 Samples should be collected in plastic or glass containers.
4.2 No preservative is required.
4.3 Mixed liquor samples should be taken at effluent end of aeration tanks.
4.4 Care should be taken to minimize floc break-up during Settlometer analysis.
4.5 Maximum holding time is 7 days.

5. Reagents

5.1 None required.

6. Equipment & Lab Ware

6.1 Imhoff Cone
6.2 Settlometer
6.3 Timer

7. Interferences

7.1 None

8. Procedures

8.1 Imhoff Cone

1) Thoroughly mix samples and fill Imhoff Cones to the 1 Liter mark.
2) Set timer for Imhoff Cone samples for 45 minutes and additional 15 minutes.
3) After the Imhoff samples have settled for 45 minutes, stir samples gently to remove suspended solids that may be on the surfaces of the Imhoff Cone.
4) After the Imhoff samples have settled for additional 15 minutes, record the amount of settled solids in mL/L.
5) Record date of sample, date analysis completed, and initial in bench sheet.

8.2 Mixed Liquor

1) Thoroughly mix sample (without aerating the sample) and fill Settlometer to the 1 Liter mark.
2) Set timer for Settlometer sample for 30 minutes, 1 hr., 2 hr., 3 hr., and 4 hr.

3) After the Settlometer sample has settled for 30 minutes, record the amount of settled solids in mL/L.
4) Observe mixed liquor throughout the 4 hour time span. If the blanket rises before 4 hours, record the rise time in the bench sheet. If after 4 hours the blanket has not risen, record >4 hours for rise time in bench sheet.
5) Record date of sample, date analysis completed and initial in bench sheet.

9. QA/QC Requirements

9.1 None Required

10. Expected Results

10.1 KPDES Permit Requirements

1) No permit requirements.

10.2 Process Ranges

Typical values (mL/L) for each plant are:

Town Branch Influent	4.74 – 10.64
Town Branch Effluent	0.02 – 0.26
West Hickman Influent	8.65 – 20.61
West Hickman Effluent	0.02 – 0.25

11. Data Analysis and Calculations

11.1 Record as milliliters per liter.
11.2 Always read the top of the solids column.
11.3 If a separation of the settleable and floating materials occurs, do not estimate the floating materials.
11.4 Detection limit: 0.01 mL/L.
11.5 % Settled Sludge = (mL of sludge in settled mixed liquor X 100)/1000

12. Bibliography

12.1 U.S. EPA Method 160.5 Residue, Settleable Matter. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

12.2 Standard Methods Part 2540-F. Settleable Solids. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

12.3 Code of Federal Regulations (CFR). Guidelines Establishing Test Procedures for the Analysis of Pollutants. 40 CFR 136.3, Title 40, Chapter 1. U.S. Environmental Protection Agency; U.S. Environmental Protection Agency. pg 5-337. 2003.

12.4 Simplified Laboratory Procedures for Wastewater Examination. Water Pollution Control Federation, Third Edition. pg 17-18. 1985.

Total Suspended Solids (TSS)
EPA Method 160.2 Residue, Non-Filterable & Total Suspended Solids
Standard Methods Part 2540-D

1. Scope, Significance to Process and Application

1.1 This method is applicable to drinking, surface, and saline waters, domestic and industrial wastes.
1.2 The practical range of the determination is 10 mg/L to 20,000 mg/L.

2. Summary of Method

2.1 Executive Summary
Total solids are the material residue left in a vessel after evaporation of a sample and subsequent oven drying at a defined temperature. Total suspended solids (TSS) is the portion of total solids that is retained by filter. Solids analyses are important in the control of biological and physical wastewater treatment processes and for assessing compliance with regulatory agency effluent limitations.

2.2 Discussion
A well-mixed sample is filtered through a standard filter, and the residue retained on the filter is dried to constant weight at 103^{o}C to 105^{o}C. The filtrate from this method may be used for Residue, Total filterable.

3. Health & Safety Precautions

3.1 Watch out for broken glass from crucibles, cylinders, and beakers.
3.2 Wastewater samples should be considered potentially hazardous. Use proper personal protective equipment.
3.3 Crucibles can be hot (103^{o}C to 105^{o}C), use proper gloves when handling.

4. Sample Handling and Preservation

4.1 Collect samples in plastic or glass containers.
4.2 No preservative required.
4.3 Maximum holding time 7 days.

5. Reagents

5.1 Drierite 8 mesh.
5.2 Nanopure Grade water.

6. Equipment & Lab Ware

6.1 Analytical Balance
6.2 ASTM Class 1 weight set
6.3 Environmental Express Pre-weighed filters for TSS (F92447MM)
6.4 Oven 103^{o}C to 105^{o}C
6.5 Beakers, stirrer, tongs
6.6 Vacuum pump and vacuum flask, 500 mL
6.7 Graduated cylinders
6.8 Desiccators

7. Interferences

7.1 Too much residue on the filter will entrap water and may require prolonged drying.
7.2 Too much residue on the filter may stop the movement of water through the filter.

8. Procedures

8.1 Steps

1) Calibrate balance using ASTM Class 1 weight set. Typically 200 g, 100 g, 500 mg, and 100 mg.

2) Environmental Express filters are pre-weighed. An ID number and weight of filter are shown on the aluminum pan. Record the ID number and the filter weight (This is W1).

3) Assemble the filtering apparatus with pre-weighed filter and apply vacuum.

4) Rinse filter with Nanopure water.

5) Mix sample.

6) Using a graduated cylinder, measure the required sample volume (V), then gently and slowly pour into filter. Rinse side of filter unit with Nanopure water.

7) Placed filter and residue back in the original aluminum pan.

8) Dry in oven (103^{o}C to 105^{o}C) until constant weight is achieved.

9) Allow pans to cool in desiccator for 20 minutes before weighing.

10) Weight of filter and residue is W2.

11) EPA requires filters to be placed back in the oven an reweighed three times.

8.2 Helpful Hints

1) Drying filters overnight produces the best results.
2) Filters stuck to the aluminum pan will produce incorrect results, re-filter sample and reweigh with a new filter, if possible.

9. QA/QC Requirements

9.1 Balance must be calibrated using ASTM Class 1 weight set before analysis.

9.2 EPA requires filters with samples be placed back in the oven an reweighed three times.

10. Expected Results

10.1 KPDES Plant Effluent Permit Requirements

1) 30 mg/L for Monthly Average
   45 mg/L for Weekly Average

In the event that analysis results indicate values greater then KPDES permit requirements, retest. If the value indicated by the retest is greater than KPDES permit requirements, **Immediately** notify the Plant Superintendent and the Laboratory Supervisor.

10.2 Process Ranges

Typical values (mg/L) for each plant are:

Town Branch Influent	89 – 485	(30 mL sample)
Town Branch Effluent	9 – 31	(500-800 mL sample)
West Hickman Influent	137 – 513	
West Hickman Effluent	2 – 72	
Blue Sky Influent	134 – 880	
Blue Sky Effluent	2 – 14	

11. Data Analysis and Calculations

11.1 Non-filterable residue, mg/L = (W2 - W1)/V*1,000,000

12. Bibliography

12.1 U.S. EPA Method 160.2 Residue, Non-Filterable & Total Suspended Solids. Issued 1971. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

12.2 Standard Methods Part 2540-D. Total Suspended Solids Dried at 103°C to 105°C. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

12.3 Code of Federal Regulations (CFR). Guidelines Establishing Test Procedures for the Analysis of Pollutants. 40 CFR 136.3, Title 40, Chapter 1. U.S. Environmental Protection Agency; U.S. Environmental Protection Agency. pg 5-337. 2003.

12.4 Simplified Laboratory Procedures for Wastewater Examination. Water Pollution Control Federation, Third Edition. pg 23-24. 1985.

12.5 Environmental Express, Inc.. http://www.envexp.com/index.asp 2009.

Total Solids
EPA Method 160.3 Residue, Total, (Gravimetric, Dried at 103 – 105 ^{o}C)
Standard Methods Part 2540-B, Total Solids Dried at 103 – 105^{o}C

1. Scope, Significance to Process and Application

1.1 This method is applicable to drinking, surface and saline waters, domestic and industrial wastes.
1.2 The practical range of the determination is from 10 mg/L to 20,000 mg/L.

2. Summary of Method

2.1 Executive Summary

Total solids are the material residue left in a vessel after evaporation of a sample and subsequent oven drying at a defined temperature. Typically, total solids include total suspended solids (TSS) and total dissolved solids (or the portion of total solids that passes through a filter). Solids analyses are important in the control of biological and physical wastewater treatment processes and for assessing compliance with regulatory agency effluent limitations.

2.2 Discussion

A well mixed aliquot of sample is quantitatively transferred to a pre-weighed evaporating dish, evaporated to dryness at 103^{o}C to 105^{o}C, and weighed to determine total solids.

3. Health & Safety Precautions

3.1 Watch out for broken glass from crucibles, cylinders, and beakers.
3.2 Wastewater samples should be considered potentially hazardous. Use proper personal protective equipment.
3.3 Crucibles can be hot (103^{o}C to 105^{o}C), use proper gloves when handling.

4. Sample Handling and Preservation

4.1 Collect samples in plastic or glass containers.
4.2 No preservative required.
4.3 Maximum holding time 7 days at 4^{o}C.

5. Reagents

5.1 Drierite 8 mesh
5.2 Nanopure Grade water

6. Equipment & Lab Ware

6.1 Denver Instrument Analytical Balance
6.2 ASTM Class 1 weight set
6.3 Vycor dishes or flat-bottom crucibles
6.4 Oven 103°C to 105°C
6.5 Plastic 150 mL beakers
6.6 Stirrers
6.7 Tongs
6.8 Desiccators

7. Interferences

7.1 Non-representative particles such as leaves, sticks, fish, and lumps of fecal matter should be excluded from the sample.
7.2 Floating oil and grease, if present, should not be included in the sample.

8. Procedures

8.1 Steps

1) Clean and dry dishes in oven overnight.

2) Remove dishes from oven and put into desiccator for at least 20 minutes.

3) Calibrate Analytical Balance using ASTM Class 1 weight set.

4) Weigh dish. This is W_1.

5) Add approximately 50 mL of mixed sample into the dish.

6) Weigh dish and sample. This is W_2.

7) Turn on fume hood.

8) Leave samples in oven overnight.

9) Remove dishes from oven and put into desiccator for at least 20 minutes.

10) The next day, calibrate Analytical Balance using ASTM Class 1 weight set.

11) Weigh dish and dry sample. This is W_3.

8.2 Helpful Hints

1) Do not allow dishes to sit out in open air before weighing.

2) Record oven temperature, date, and time. Make sure to initial records.

9. QA/QC Requirements

9.1 None required

10. Expected Results

10.1 KPDES Permit Requirements

1) None required.

10.2 Process Ranges

Typical values (% total solids, mg/L) for each plant are:

Town Branch

W.A.S. Thickener	3.68
Raw Sludge to Thickener	0.41 – 0.97
Digested Sludge	1.49 – 1.84
Belt Press Cakes	15.35 – 19.68

West Hickman

Return Activated Sludge	0.79 – 1.16
W.A.S. to Thickener	0.79 – 1.16
Belt Press Cakes	16.45 – 18.94

11. Data Analysis and Calculations

11.1 Total Solids mg/L = $(W_3 – W_1)/(W_2 – W_1)*1{,}000{,}000$

11.2 % Total Solids mg/L = $(W_3 – W_1)/(W_2 – W_1)*100$

12. Bibliography

12.1 U.S. EPA Method 160.3 Residue, Total (Gravimetric, Dried at 103 – 105^{o}C). Issued 1971. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

12.2 Standard Methods Part 2540-B. Total Solids dried at 103^{o}C to 105^{o}C. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

12.3 Code of Federal Regulations (CFR). Guidelines Establishing Test Procedures for the Analysis of Pollutants. 40 CFR 136.3, Title 40, Chapter 1. U.S. Environmental Protection Agency; U.S. Environmental Protection Agency. pg 5-337. 2003.

12.4 Simplified Laboratory Procedures for Wastewater Examination. Water Pollution Control Federation, Third Edition. pg 25-27. 1985.

Volatile Acids
Standard Methods Part 5560-C Distillation Method
HACH Method 8291, Buret Titration Method

1. Scope, Significance to Process and Application

1.1 This technique recovers acids containing up to six carbon atoms.
1.2 Fractional recovery of each acid increases with increasing molecular weight.
1.3 Calculations and reporting are on the basis of acetic acid.
1.4 The method is often applicable for control purposes.

2. Summary of Method

2.1 Executive Summary

Volatile fatty acids are classified as water-soluble fatty acids (up to six carbon atoms) that can be distilled at atmospheric pressure because of co-distillation with water. A sample is acidified with sulfuric acid, distilled, and the distillate is titrated to the phenolphthalein end point with sodium hydroxide. The volume of titrant is proportional to the volatile acids concentration. Results are expressed as mg/L as acetic acid.

2.2 Discussion

Acidity of water is its quantitative capacity to react with a strong base to a designated pH. Acidity is a measure of an aggregate property of water and can be interpreted in terms of specific substances only when the chemical composition of the sample is known. In this case, 125 mL is distilled, brought to 95^{o}C, and titrated until a slight pinkish color is obtained.

3. Health & Safety Precautions

3.1 Watch out for broken glass from cylinders and beakers.
3.2 Wastewater samples should be considered potentially hazardous. Use proper personal protective equipment.
3.3 Distillation flasks can be HOT, use proper gloves when handling.
3.4 Mercury from broken thermometers can be a safety hazard.
3.5 Sulfuric Acid is used during this analysis. Wear gloves, goggles and lab coat.
3.6 ALWAYS ADD ACID TO WATER - NOT WATER TO ACID!!!

4. Sample Handling and Preservation

4.1 None required.

5. Reagents

5.1 Sulfuric acid 1:1
5.2 0.1 N Sodium hydroxide
5.3 Phenolphthalein Indicator
5.4 Nanopure Water
5.5 Glacial acetic acid

6. Equipment & Lab Ware

6.1 Heating Mantles
6.2 Hot plate
6.3 Centrifuge tubes
6.4 Boiling flask
6.5 Condenser, about 76 cm long
6.6 Distillation Assembly
6.7 Adapter tubes
6.8 Erlenmeyer flasks
6.9 Thermometers with stoppers
6.10 Titrator
6.11 Digital Buret
6.12 Plastic funnels
6.13 5 mL pipette and tips
6.14 Timer
6.15 Finger, vinyl, leather gloves
6.16 100 mL & 20 mL graduated cylinders

7. Interferences

7.1 Hydrogen sulfide and carbon dioxide are liberated during distillation and can be titrated to give a positive error. Eliminate error by discarding the first 15 mL of distillate and account for this in the recovery factor.

8. Procedures

8.1 Steps

1) Turn on heating mantles.

2) Make sure hot plate is on.

3) Fill centrifuge tubes to obtain 125 mL of sample. Centrifuge samples for 15 minutes.

4) Gently swirl tubes to mix, and centrifuge for an additional 15 minutes.

5) Decant 125 mL of sample and pour into the Boiling flask.

6) Add 125 mL of Nanopure water to the Boiling flask. Swirl to mix.

7) Add 5 mL of 1:1 Sulfuric Acid to the Boiling flask. Swirl to mix

8) Connect flask to a condenser and adapter tube.

9) Distill at a rate of 5 mL/min.

10) Make sure tap water is running and cooling the condenser.

11) Set timer for 5, 20, and 20 more minutes.

12) Collect the first 15 mL of distillate in 20 mL graduated cylinder and discard. This takes approximately 5 minutes.

13) Collect 150 mL of distillate in an Erlenmeyer flask. This takes approximately 20 minutes.

14) While wearing leather gloves, disconnect Boiling flask from distillation unit.

15) Transfer the Erlenmeyer flask to the hot plate, insert thermometer, and stopper.

16) Heat to $90^{o}C \pm 5^{o}C$. This takes approximately 20 minutes.

17) While wearing finger gloves, remove flask from hot plate.

18) Add 10 drops of Phenolphthalein Indicator. Swirl to mix.

19) Titrate, drop-wise, with 0.1 N Sodium hydroxide (NaOH) using the Brand Digital Buret II until first persistent pink color is obtained.

20) Record in bench sheet end point, date sample collected, date analysis performed, time analysis started, and initials.

8.2 Recovery Factor Determination

To determine the recovery factor (f):

1) Dilute 1.9 mL of glacial acetic acid in 1 L Nanopure water (2000 mg/L acetic acid solution).

2) Add 50 mL of prepared acetic acid solution to 1 L volumetric flask and bring to 1 L with Nanopure water (final acetic acid concentration = 100 mg/L).

3) Measure 250 mL of diluted acetic acid solution and distill as shown in Section 8.1.

4) Titrate drop wise with 0.1 N Sodium Hydroxide (NaOH) using the Brand Digital Buret II until first persistent pink color is obtained.

5) Calculations:

a = (volume of NaOH required)(0.1N)(60000)/250 mL

Recovery factor (f) = a/b , where b=100 mg/L

8.3 Helpful Hints

1) If there is no color change, it means no Sulfuric acid was added.

9. QA/QC Requirements

9.1 None required.

10. Expected Results

10.1 KPDES Permit Requirements

1) None required.

10.2 Process Ranges

1) Town Branch Digesters ranges are 35 – 73 mg/L.
2) If volatile acids ratio is high for plant operations, run analysis again.

11. Data Analysis and Calculations

11.1 mg volatile acids as acetic acid/L = (mL NaOH)(0.1N)(60,000)/ (mL sample)(f)
Where: f = recovery factor

12. Bibliography

12.1 Standard Methods Part 5560-C, Distillation Method. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

12.2 HACH Water Analysis Handbook. Method 8291, Volatile Acids, Buret Titration Method. 2nd Edition. HACH Company, Loveland, CO, 1992.

Volatile Solids
EPA Method 160.4 Residue, Volatile, (Gravimetric, Ignition at 550°C), Issued 1971
Standard Methods Part 2540-E, Fixed and Volatile Solids Ignited at 550°C

1. Scope, Significance to Process and Application

1.1 This method is applicable to drinking, surface and saline waters, domestic and industrial wastes.

2. Summary of Method

2.1 Executive Summary

Total solids are the material residue left in a vessel after evaporation of a sample and subsequent oven drying at a defined temperature. The total solids are then ignited to a constant weight at 550°C and the weight loss on ignition represents the volatile solids. Volatile solids analyses are important because they offer a rough approximation of the amount of organic matter present in the solid fraction of wastewater, activated sludge, and industrial waste.

2.2 Discussion

Volatile solids are the weight lost due to ignition. The remaining solids represent the fixed total.

3. Health & Safety Precautions

3.1 Watch out for broken glass from Vycor dishes, cylinders, and beakers.
3.2 Wastewater samples should be considered potentially hazardous. Use proper personal protective equipment.
3.3 Dishes are HOT (550°C), use proper gloves when handling. Watch out for hot surfaces.

4. Sample Handling and Preservation

4.1 Do immediately after Total Solids Method (See SOP - Total Solids).

5. Reagents

5.1 Drierite 8 mesh.

6. Equipment & Lab Ware

6.1 Denver Instrument Analytical Balance
6.2 ASTM Class 1 weight set
6.3 Vycor dishes or flat bottom crucibles
6.4 Tongs
6.5 Thermolyne 30400 Muffle Furnace (550^{o}C)
6.6 Desiccator
6.7 Hot Plate (103^{o}C to 105^{o}C)

7. Interferences

7.1 Negative errors in the volatile solids may be produced by loss of volatile matter during drying.
7.2 Determination of low concentrations of volatile solids in the presence of high fixed solids concentrations may be subject to considerable error.

8. Procedures

8.1 Steps

1) After Total Solids Method is completed (See SOP - Total Solids) place dishes in Muffle Furnace. Use tongs and gloves.

2) Ignite tares at 550 ± 50^{o}C to constant weight (approximately 1 hr).

3) Transfer tares to hot plate for 15 minutes.

4) Transfer tares to desiccator for at least 20 minutes. (Note: Make sure the dishes are cool enough not to melt the desiccator).

5) Weigh tare and ash and record weight as W_4.

8.2 Helpful Hints
1) Transfer tares to hot plate so it doesn't melt desiccator.
2) Radiant heat from furnace can burn, wear gloves and use tongs.

9. QA/QC Requirements

9.1 None required.

10. Expected Results

10.1 KPDES Permit Requirements
None required.

If value is inconsistent, DNS (Data Not Supportable) is typed into computer.

10.2 Process Ranges
The practical determination range is 10 mg/L to 20,000 mg/L.

11. Data Analysis and Calculations

11.1 Volatile Solids (mg/L) = $(W_3 – W_4)/ (W_2 – W_1) * 1{,}000{,}000$
11.2 % Volatile Solids = $(W_3 – W_4)/ (W_3 – W_1) * 100$

12. Bibliography

12.1 U.S. EPA Method 160.4 Residue, Volatile, (Gravimetric, Ignition at 550^{o}C), Issued 1971. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

12.2 Standard Methods Part 2540-E, Fixed and Volatile Solids Ignited at 550^{o}C. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

WEST HICKMAN CREEK SOPs

WH Dissolved Oxygen Field Analysis (D.O.)
HACH Method 10360 Luminescent Dissolved Oxygen Probe Method
Proposed EPA Method 360.3 (Luminescence) for the Measurement of Dissolved Oxygen in Water and Wastewater

1. Scope, Significance to Process and Application

1.1 Dissolved Oxygen (D.O.) analysis measures the concentration of oxygen that is dissolved in a water sample.

1.2 This method is recommended for samples containing intense color or turbidity which interferes with the Winkler method.

1.3 This method is recommended for work in the field, as the equipment is portable, allowing hold times to be minimized.

1.4 KPDES Permit Limits on Plant Effluent Required a minimum of 7.0 mg/L.

1.5 Dissolved Oxygen concentration levels are very important in both process and plant effluents. In process, dissolved oxygen is required by various organisms and the plant effluent dissolved oxygen levels must be conducive to the receiving environment and within permit limits (See Section 10.1).

2. Summary of Method

2.1 Executive Summary

Dissolved Oxygen is measured directly by a HACH model HQ40d portable meter and HACH model LDO101 rugged field dissolved oxygen probe. After the meter indicates a stable reading the operator/analyst records the value.

2.2 Discussion

The HACH LDO system uses a sensor coated with a luminescent material. Blue light from an LED is transmitted onto the sensor surface, exciting the luminescent material, which then emits red light as it relaxes. The presence of DO in the process shortens the time it takes for the red light to be emitted. By measuring the time lapse between when the blue light was transmitted and the red light is emitted, a correlation is made to the concentration of DO in the effluent or other solution. Between measurements, a red LED is used as an internal reference. The measurement range for the method is 0.02 mg/L to 20.0 mg/L. The Method Detection Limit (MDL; 40 CFR 136, Appendix B) has been determined as 0.05 mg/L and the Minimum Level (ML; Reference 15.4) has been set at 0.20 mg/L.

3. Health & Safety Precautions

3.1 Glassware involved: possible cut hazard.
3.2 All municipal and industrial wastewaters are potentially hazardous. Gloves and goggles should be worn when dispensing these samples.

4. Sample Handling and Preservation

4.1 Field measurements are obtained directly, therefore, sample handling may not apply.
4.2 If samples are collected for analysis at another location (i.e. Laboratory), the following apply:

4.2.1 Sample must be collected in a glass bottle (preferably a BOD bottle with stopper) filled to top, with no airspace.
4.2.2 Sample must be analyzed immediately (15 minutes maximum on permit samples).

4.3 There is no applicable preservative with this method.

5. Reagents

5.1 Nanopure Lab Water

6. Equipment & Lab Ware

6.1 HACH HQ40d portable multi-meter
6.2 HACH Model LDO101 Rugged Dissolved Oxygen Probe
6.3 BOD bottles with 300 ml capacity and tapered ground glass stoppers
6.4 Sensor Cap replacements (HACH part # 5838000)
6.5 Calibration bottle for "Water-saturated Air" calibration method

7. Interferences

7.1 Salinity (salinity correction available, See Section 8.4.3 of the Users Manual).
7.2 Reactive gas: chlorine and hydrogen sulfide.
7.3 Air bubbles in sample or on surface of probe tip.

8. Procedures

8.1 Calibration and Start Up

It is suggested that at the Users Manual be initially consulted when following these procedures.

1) Press the power button on the HQ40d and allow the unit to perform its startup self check routine.

2) Clean by rinsing with Nanopure lab water, then blot dry the probes tip with a Kim-Wipe. Inspect the probe tip for indications contamination or damage.

3) Take a 300 mL BOD bottle containing approximately one inch of lab water, cap and shake, remove cap and replace it with the probe.

4) Press Calibrate (blue button), the meter will prompt you to "Dry the probe and place in water saturated air & press "Read". Press "Read", the screen will scroll from 0 to 100%, then indicate "Calibration Complete". Record from display screen both the temperature and the dissolved oxygen value (indicated under the temperature). Log the values on the dissolved oxygen calibration section of the Dissolved Oxygen bench sheet under D=Temperature and E=Dissolved Oxygen from HQ40d.

5) Note the barometric pressure value from the laboratory barometer (located adjacent to the D.O. meter) and record on the dissolved oxygen calibration section of the Dissolved Oxygen bench sheet under "Barometer Reading".

6) On the Lab computer, open the excel spreadsheet entitled "DO Meter Calibration Sheet" and enter the barometric pressure, temperature and dissolved oxygen values from the dissolved oxygen calibration section of the Dissolved Oxygen bench sheet. The spreadsheet will calculate the "Dissolved Oxygen Calibration Point", the "Dissolved Oxygen @ 1 ATM" and the Slope %. Transfer the three values onto the dissolved oxygen calibration section of the Dissolved Oxygen bench sheet, then print a copy of the spreadsheet and file it.

7) Note the difference between the Dissolved Oxygen from HQ40d and the Dissolved Oxygen Calibration Point - if it is greater than 0.2 mg/L, then the calibration is not acceptable and must be repeated until criteria is met.

8.2 Measurements

1) Make sure that the meter is properly calibrated.

2) Rinse the LDO101 probe tip with Nanopure lab water.

3) Place probe into a BOD bottle filled to the base of its neck with sample, assure that there are no air bubbles on the surface of the probe tip. In the field, the probe is lowered into the sample.

4) Press Read, screen will display "Stabilizing" and a progress bar will scroll from 0 to 100%.

5) Reading stability is indicated by the appearance of a "Padlock" icon in the upper left corner of the display screen. Record the indicated value, remove the probe, and rinse tip with Nanopure water. Then proceed to the next sample or store until needed.

6) DO NOT store probe in the BOD bottle containing water. Probe can be stored dry on the bench top.

8.3 Helpful Hints

1) The meter is designed to be maintenance free, when needed, clean the exterior with a damp cloth.

2) The probe's maintenance consist of maintaining the probe tip clean, frequent rinsing with Nanopure water is sufficient.

3) DO NOT scrub the sensor cap or lens.

4) DO NOT use any organic solvents on the sensor cap or probe body.

9.0 QA/QC Requirements

9.1 Meter must be calibrated a minimum of once per analysis day.
9.2 Permit sample hold times must be 15 minutes or less.
9.3 Probe condition must be properly maintained through routine cleaning (See Section 8.3, Helpful Hints).

10. Expected Results

10.1 KPDES Permit Requirements
7.0 mg/L is the lowest D.O. value allowable in a plant effluent sample at any given time. In the event of an indicated value less than 7.0 mg/L, assure correct calibration, resample, and retest. If the value indicated by retest is less than 7.0 mg/L, **Immediately** notify the Plant Superintendent and the Laboratory Supervisor.

10.2 Process Ranges
Raw influent dissolved oxygen values are typically less than 1 mg/L.
Target values for mixed liquor dissolved oxygen concentration in the aeration basins is 2.0 mg/L.
Plant effluent dissolved oxygen values must be maintained at or above 7.0 mg/L.

11. Data Analysis and Calculations

11.1 None required, values are taken directly when measurement stability is indicated.

12. Bibliography

12.1 Report on the Validation of Proposed EPA Method 360.3 (Luminescence) for the Measurement of Dissolved Oxygen in Water and Wastewater. August 2004. HACH Company, Loveland, CO, 2004.

12.2 Memorandum: EPA Recommendation for the use of HACH method 10360 [Revision 1.1, January 2006] (ATP Case # N04-0013).

12.3 HACH HQ Series Portable Meter Users Manual, September 2006, Edition 5. HACH Company, Loveland, CO, 2006.

WH pH (Electrometric)
EPA Method 150.1 pH. Issued 1971 (Editorial revision 1978 and 1982)
HACH USEPA Electrode Method 8156

1. Scope, Significance to Process and Application

1.1 At a given temperature the intensity of the acidic or basic character of a solution is indicated by a pH or hydrogen ion activity.
1.2 A pH meter is accurate and reproducible to 0.1 pH unit with a range of 0 to 14 and equipped with a temperature compensation adjustment.

2. Summary of Method

2.1 Executive Summary
The pH of a sample is determined electrometrically using either a glass electrode in combination with a reference potential or a combination electrode.

2.2 Discussion
There is no color change during analysis. pH stands for power of hydrogen, a measure of hydrogen ion concentration in solution.

3. Health & Safety Precautions

3.1 Watch out for broken glass from beakers and cylinders.
3.2 Wastewater samples should be considered potentially hazardous. Use proper personal protective equipment.

4. Sample Handling and Preservation

4.1 Samples should be analyzed as soon as possible within a 15 minute time window, preferably in the field.
4.2 Collect samples in clean plastic or glass bottles. Sample containers should be filled completely and kept sealed prior to analysis.

5. Reagents

5.1 Buffer Solution pH – 4.00 (color coded red)
5.2 Buffer Solution pH – 7.00 (color coded yellow)
5.3 Buffer Solution pH – 10.00 (color coded blue)
5.4 Nanopure Grade Laboratory Water
5.5 pH Electrode Storage Solution

6. Equipment & Lab Ware

6.1 HQ40d Dual-Input Multi-Parameter Digital Meter
6.2 PHC101 IntelliCAL Rugged Gel Filled pH Electrode

7. Interferences

7.1 pH measurements are affected by temperature and can cause the reading to drift.
7.2 Grease and other debris on the probe can cause inaccurate readings.

8. Procedures

8.1 Calibration

1) Refer to the operation section of the electrode or meter manual to prepare the PHC101 pH electrode and HQ40d meter.

2) Turn the HQ40d meter on. Push the "Down" arrow.

3) Make sure that the meter is set to measure to measure pH.

4) In three separate beakers or appropriate containers, prepare fresh buffers of pH 7.0, 4.0 and 10.0.

5) Place probe in pH 7.0 buffer (yellow color coded).

6) Turn on stir plate.

7) Press READ and wait until a stable reading is obtained (lock icon).

8) Rinse probe with Nanopure water.

9) Place probe in pH 4.0 buffer (red color coded).

10) Turn on stir plate.

11) Press READ and wait until a stable reading is obtained (lock icon).

12) Rinse probe with Nanopure water.

13) Place probe in pH 10.0 buffer (blue color coded).

14) Turn on stir plate.

15) Press READ and wait until a stable reading is obtained (lock icon).

16) Rinse probe with Nanopure water.

17) Press DONE and the meter will display all data gathered.

18) Make sure that the calibration slope is acceptable (typically -58 ±3 mV per pH unit at 25°C).

19) Press STORE twice to accept calibration.

20) Rinse the electrode in Nanopure water and blot dry prior to sample measurement. Rinse the electrode with Nanopure between measurements to minimize contamination.

8.2 Sample Measurement

1) Put the electrode in the sample. In the field, readings are taken directly.

2) Turn on stir plate and press READ. For faster response, stir at a slow to moderate rate.

3) When the measurement is stable, store or record the pH and temperature values. For the HQ40d meter, data is stored automatically when Press to Read or Interval is selected in the Setup Measurement Mode. When Continuous is selected, data will only be stored when the key under STORE is pressed.

4) Store the pH electrode in pH storage solution when not in use. See Sample collection, preservation, general storage and cleaning for more details

8.3 Helpful Hints

1) Avoid strong acids and greasy samples.

2) Make sure that probe is thoroughly rinsed between samples so that cross contamination does not occur.

3) Storage of an electrode is based on how long the electrode will be stored, how quickly the electrode needs to be used and the type of sample being measured. For general storage, use the HACH storage solution or a 3M Potassium chloride (KCl) solution.

4) A contaminated glass bulb or fouled electrode may cause slow response times. Do not clean the bulb too often because the bulb life may shorten.

5) To clean an electrode with general contamination, immerse the electrode tip in 0.1N Hydrochloric acid (HCl). Then, immerse the electrode in 0.1N Sodium hydroxide (NaOH) and again in 0.1N Hydrochloric acid, each for a 2-minute period. Rinse with Nanopure water and soak in Nanopure water for at least 15 minutes.

6) To clean an electrode contaminated with oils and fats, immerse the electrode tip in a detergent solution. Use a soft brush or ultrasonic bath if necessary. Avoid scratching the glass bulb.

9. QA/QC Requirements

9.1 If sample is not collected properly or analyzed within 15 minutes, another sample must be obtained and analyzed.

9.2 **Check electrode response**
An electrode is responding properly if its calibration slope meets the slope specifications of the electrode (typically -58 ±3 mV at 25°C).

9.3 **Check calibration accuracy**
Return the electrode to a calibration buffer and measure the pH to test the system. Rinse and recondition the electrode before measuring subsequent samples.

10. Expected Results

10.1 KPDES Permit Requirements
1) 6.0 mg/L is the lowest pH allowed.
2) 9.0 mg/L is the highest pH allowed.

In the event that analysis results indicate values greater then KPDES permit requirements, retest. If the value indicated by the retest is greater than KPDES permit requirements, **Immediately** notify the Plant Superintendent and the Laboratory Supervisor.

10.2 Process Ranges

Typical values for each plant are:

Town Branch Influent	7.3 – 7.4
Town Branch Effluent	6.7 – 8.9
West Hickman Influent	7.1 – 7.4
West Hickman Effluent	6.4 – 7.7
Blue Sky Influent	7.1 – 7.5
Blue Sky Effluent	6.0 – 7.3

11. Data Analysis and Calculations

11.1 None required.

12. Bibliography

12.1 U.S. EPA. Method 150.1 pH (Electrometric) Issued 1971 (Editorial revision 1978 and 1982). Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020; U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

12.2 HACH. USEPA Electrode Method 8156. DOC316.53.01245. Edition 5. HACH Company, Loveland, CO, 2008.

12.3 Code of Federal Regulations (CFR). Guidelines Establishing Test Procedures for the Analysis of Pollutants. 40 CFR 136.3, Title 40, Chapter 1. U.S. Environmental Protection Agency; U.S. Environmental Protection Agency. pg 5-337. 2003.

WH Total Residual Chlorine Analysis
HACH AutoCAT 9000 Total Chlorine Amperometric Forward Titration
procedure equivalent to
EPA Method 330.1 Chlorine, Total Residual (Titrimetric, Amperometric), Issued 1978
Standard Methods Part 4500-Cl D. (Chlorine Residual Amperometric Titration Method)

1. Scope, Significance to Process and Application

1.1 Disinfection by chlorination is considered to be the primary mechanism for the inactivation/destruction of pathogenic organisms in wastewater treatment plant effluents and to prevent the spread of waterborne diseases to downstream users and the environment. Final clarifier effluent is treated with chlorine as enters the chlorine contact tanks allowing contact time for disinfection to transpire. Final effluent is then treated with a dechlorinating agent reducing chlorine residual concentration to within acceptable limits (see sec. 10.1 Permit limits). Complete dechlorination is necessary to prevent chlorine related adverse effects on the receiving environment. Town Branch Waste Water Treatment Plant uses Chlorine Dioxide (ClO_2) for chlorination and Sulfur Dioxide (SO_2) as the dechlorinating agent. Residual Chlorine analysis of treated plant effluent validates efficacy of dechlorinating agent dosing and permit compliance.

2. Summary of Method

2.1 Executive Summary

West Hickman Laboratory uses a HACH AutoCAT 9000 autotitrator to perform Residual Chlorine determinations. The AutoCAT 9000 bench top system automatically completes all USEPA- approved amperometric titration methods for chlorine, calculates analyte concentration, and provides real-time graphics display. The AutoCAT's forward amperometric titration procedure has a range of 0.0012 mg/L to 5.0 mg/L with an estimated detection limit of 0.0012 mg/L

2.2 Discussion

Chlorine (hypochlorite ion, hypochlorous acid) and chloramines liberate iodine from potassium iodide at pH 4 or less in stoichiometeric proportions. The iodine is titrated with a reducing agent phenylarsine and an amperometer detects the endpoint. Although the actual measurement is that of the samples oxidation potential, it is calculated and expressed as mg/L Cl because chlorine is the dominating oxidizing agent present.

3. Health & Safety Precautions

3.1 Glassware involved, possible cut hazard.
3.2 Wastewater samples have the potential to be hazardous, use appropriate caution.

4. Sample Handling and Preservation

4.1 Residual chlorine is subject to dissipation by exposure to sunlight, mechanical agitation, exchange of gases with the atmosphere and reaction with compounds in the wastewater over time. For these reasons chlorine residuals should be analyzed immediately (within 15 minutes of sampling time).

4.2 The sample should be taken gently into a glass 300 mL BOD bottle, completely filling to above the base of the neck and installing the tapered glass stopper in a manner that precludes air bubbles in the sample.

4.3 All glassware used in this method must have no chlorine demand, therefore do not use plastic containers and pre-treat glassware accordingly. To remove chlorine demand from clean glassware, soak in a dilute bleach solution (1 mL commercial bleach to 1 liter of Nanopure water) for at least one hour. After soaking, rinse thoroughly with Nanopure water. After analysis, thoroughly rinse all glassware with Nanopure water to reduce the need for pretreatment.

5. Reagents

5.1 Reagent 1 - Potassium Iodide 5%

5.2 Reagent 2 - pH 4.00 Buffer (Certified Acetic Acid). Both reagents are located adjacent to the AutoCAT unit

5.3 Phenylarsine Oxide Standard Solution 0.00564 N

5.4 Chlorine Standard Solution, 25-30 mg/L as Cl_2

6. Equipment & Lab Ware

6.1 HACH AutoCAT 9000 - Chlorine Amperometric Titrator

6.2 Beakers 250 mL

6.3 Graduated Cylinders 250 mL

6.4 1 mL fixed volume Finnpipette and 1 mL tips

6.5 Stirring bars.

7. Interferences

7.1 Accurate determinations of free chlorine cannot be made in the presence of Nitrogen trichloride or Chlorine dioxide.

7.2 Some organic chloramines can also interfere.

7.3 Free halogens other than chlorine also will titrate as free chlorine.

7.4 Interference from copper has been noted in samples taken from copper pipe or after heavy copper sulfate treatment of reservoirs.

7.5 Contamination of probe by metal ions such as copper, silver, iron interfere with amperometric titrations. Fouled electrodes will not produce sharp endpoints.

7.6 Extended sample hold times, volatilization from mechanical agitation, and exposure to various light sources can affect results.

7.7 At very low temperatures, there is slow response of cell and longer time is required, but precision is not compromised.

8. Procedures

8.1 Steps

1) Prior to testing, pre-rinse all glassware and stir bars with sample (Do not rinse with Nanopure once pre-rinsed).
2) Using a 250 mL graduated cylinder measure 200 mL of sample.
3) Pour sample into a 250 mL beaker with stirring bar, raise the electrode assembly and place the beaker on unit.
4) Turn on instrument. The display will request user to press "1" for "Total Cl_2 Fwd", press "1", display will then request user confirmation, press "1" to confirm.
5) The display will request confirmation of sample volume (200 mL) press "1"
6) The sample will begin to stir.
7) Display will request the addition of 1 mL of Potassium Iodide 5% (Reagent 1), pipette reagent into sample, then press "OK"
8) Display will request the addition of 1 mL of Acetate buffer pH 4 (Reagent 2), pipette reagent into sample, then press "OK"
9) A mixing timer will countdown for 5 sec. then the display will request that the electrodes be dipped into the sample, lower electrode assembly into sample and press "OK".
10) The display will request user to confirm the "Increment Setting" (should be 0.0010), press "1" to confirm.
11) Unit will begin analysis; total time required for analysis will vary with sample strength and chosen increment value. During analysis the display graphs the progress of the titration. Upon completion the unit will display the resulting concentration value and calculated confidence limits, press "OK" to accept results.
12) Record the results on the Total Chlorine Residual bench sheet. In the case of the PTE sample, also log (in the provided location) the time sampled, time received, and time analysis began. Note: If sample hold time (time sampled to time analysis begins) exceeds 15 minutes the analysis is void and must be rerun, beginning with resampling.
13) Select "END" if done with analysis or "Continue" to proceed to the next sample to be analyzed.

Note: More detailed general information on the AutoCAT unit can be found in the operator's manual with details on the Forward Amperometric procedure starting on page 101. The manual is located on the shelf adjacent to the AutoCAT unit.

8.2 Chlorine Standard Analysis

1) Obtain a Chlorine Standard Solution (25-30 mg/L) ampoule from "Chemical Storage" fridge.
2) Carefully break top off ampoule.
3) Pipette 1.0 mL of standard into 1000 mL volumetric flask.
4) Bring to 1000 mL with Nanopure water.
5) Measure 200 mL of standard solution into beaker with stir bar.
6) Run titration as indicated in Section 8.1.
7) Record results in bench sheet.
8) The Laboratory Supervisor will determine if the standard is within the expected range.
9) Measure 200 mL of Nanopure water into a clean 250 mL beaker and analyze as indicated in Section 8.1 (This will be a Blank to confirm no chlorine carry-over). Record results in bench sheet.
10) If chlorine is detected, re-run Blank until Below Detection Limit (BDL) is obtained.

8.3 Helpful Hints

1) Analysis results are directly proportional to sample volumes therefore it is very important that sample volume measurement is accurate.
2) Clean conditioned electrodes are required for the production of sharp, well defined endpoints that are needed for precise analysis. Rinse electrodes thoroughly before and after each use with Nanopure water, and store in Nanopure water.
3) Routine use of the "Electrode Cleaning and Conditioning" procedure as described in Section 9.1.4 of the Operator's Manual will prevent problems.
4) Glassware must be clean and free of chlorine demand see section 4.3

9. QA/QC Requirements

9.1 A diluted standard (25-30 mg/L) and Blank(s) must be run once a week (See Section 8.2).

9.2 5% of all samples must be run in duplicate.

9.3 Data acceptance criteria:

1) Results for the Standard must agree within 10% of the standard's known value.
2) Duplicate values must agree within 5%.
3) If these criteria are not met, corrective action is indicated. See Quality Assurance Program (QAP) Sec. 15 "Corrective Action Policies and Procedures".

10. Expected Results

10.1 KPDES Permit Requirements
KPDES Permit Limits on plant effluent residual chlorine at West Hickman WWTP is a maximum monthly average of 0.011 mg/L, with a daily maximum limitation of 0.019 mg/L. In the event that analysis results indicate values greater then KPDES permit requirements, retest. If the value indicated by the retest is greater than KPDES permit requirements, **Immediately** notify the Plant Superintendent and Laboratory Supervisor.

10.2 Process Ranges
Expected residual chlorine results on plant effluent samples will be less than 0.010 mg/L, typically the results are BDL (below detection limit).

11. Data Analysis and Calculations

11.1 Concentration values are read directly from the AutoCAT unit's display, all calculations are preformed internally.
11.2 The Laboratory Supervisor will determine if the results for the standard are within the expected range.

12. Bibliography

12.1 U.S. EPA Method 330.3 Chlorine, Total Residual (Titrimetric, Amperometric) Issued 1978. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

12.2 Standard Methods 4500-Cl D. Chlorine Residual Amperometric Titration Method. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

12.3 HACH AutoCat 9000 Chlorine Amperometric Titrator Instruction Manual. HACH Company, Loveland, CO.

WH Fecal Coliform - Membrane Filter Procedure
U.S. EPA 600/8-78-017 Microbiological Methods for Monitoring the Environment: Water and Wastes

1. Scope, Significance to Process and Application

1.1 The fecal coliform analysis is applicable to investigations of stream pollution, raw water sources, and wastewater treatment systems.

1.2 The fecal coliform analysis differentiates between coliforms of fecal origin.

2. Summary of Method

2.1 Executive Summary

The sample is filtered through a Millipore® membrane filter. The filter is placed on a filter pad containing media in a sterile Petri dish. The samples are then incubated at 44.5°C ± 0.2°C for 24 hours ± 2 hours. Colonies are counted and fecal coliform calculations are performed.

2.2 Discussion

Fecal coliforms are defined as gram-negative, non-spore forming rods. The major species is *Escherichia coli*, which indicates fecal pollution and the presence of enteric pathogens. Colonies produced by fecal coliform bacteria are various shades of blue. Non-fecal coliform colonies are gray to cream colored.

3. Health & Safety Precautions

3.1 All municipal and industrial wastewaters are potentially hazardous. Gloves and safety glasses should be worn when dispensing these samples.
3.2 Possible exposure to enteric pathogens. Care must be taken to avoid undue exposure.
3.3 A flame is used to sterilize forceps. Maintain the area around the flame clear.
3.4 Contaminated (used) Petri dishes and lab equipment must be placed in Biohazardous waste container. This Biohazardous waste container is autoclaved before disposal.

4. Sample Handling and Preservation

4.1 Samples should be collected in clean, sterile glass or plastic containers.
4.2 If chlorine is in the sample, containers should be treated with 4 drops of 10% Sodium thiosulfate before autoclaving.
4.3 Run test immediately after sampling, or preserve sample at 4°C for a maximum of 6 hours.

5. Reagents

5.1 Peptone powder
5.2 Peptone buffer solution pH 7.00 ± 0.1 (stored at 4°C)
5.3 m-FC media with rosolic acid for fecal coliforms (stored at 4°C)
5.4 Sodium thiosulfate 10% solution
5.5 Lysol disinfectant, 20% solution

6. Equipment & Lab Ware

6.1 Vacuum flask
6.2 Millipore® single use 47 mm Petri dishes with pads
6.3 Millipore® sterilized 47 mm filter
6.4 Forceps
6.5 4.5 X 9 inch sterile sampling bags
6.6 Bunsen burner and striker
6.7 Pipettes and sterile tips
6.8 Sterilized filter holder (plastic or glass)
6.9 Gable topped water bath at 44.5°C ± 0.2°C
6.10 Thermometer
6.11 ASTM Thermometer
6.12 Tower Steam Indicator Strips
6.13 ODO-Clave® Heat Activated Deodorant Pads
6.14 Autoclavable Biohazard waste bags and deposit box
6.15 Autoclave
6.16 Sterile blue sheets
6.17 Indicator tape
6.18 Autoclavable Nalgene® squeeze bottles for peptone

7. Interferences

7.1 Bacteria from the surrounding environment.
7.2 Cross contamination from one sample to the next.
7.3 Lack of aseptic techniques.

8. Procedures

8.1 Steps

1) Clean work area with Lysol disinfectant, 20% solution.

2) Light Bunsen burner with striker.

3) Open sterile filter holder. Use sterile blue sheet as a sterile field. Indicator trip and tape should indicate that the filter has been sterilized.

4) Flame forceps and use it to remove the Indicator Strip without touching anything else except the strip.

5) Use Petri dishes with sterile pad already in dish.

6) Break open ampule of media and pour onto media pad.

7) Decant excess media and cover dish to protect sterile pad.

8) Place the bottom of the sterile filter holder onto the vacuum flask.

9) Flame forceps, remove sterilized filter from packaging and place onto sterilized filter holder (grid side up). Do not touch the filter with anything except the forceps.

10) Place or clamp the top unit onto filter holder.

11) Gently mix sample.

12) In advance, determine sample volume that will yield 20-60 fecal coliform units (FCU).

13) If the volume of sample to be used is 0.1 to 5 mL, pour approximately 10 mL of peptone into filter unit before dispensing sample (Turn on vacuum **after** the sample is introduced).

14) For sample volumes 5 to 50 mL, use sterile pipettes for dispensing into filter unit.

15) Do not touch the inside of the filter holder unit. Do not allow the pipette tip to touch the filter.

16) For sample volumes 50 to 100 mL, pour sample into cylinder and use the scale on the side of the cylinder for measurement.

17) Turn on vacuum.

18) Once sample has filtered through, turn off vacuum.

19) Rinse top of the filter unit with peptone two times using autoclaved peptone in a Nalgene® squeeze bottle.

20) Turn on vacuum to drain peptone.

21) Turn off vacuum.

22) Flame forceps.

23) Remove top of the filter unit and place on the sterile blue field.

24) Open Petri dish.

25) Use sterile (flamed) forceps to grab the edge of the filter and remove it from the filter holder unit.

26) Place filter, grid side up, onto edge of the Petri dish and gently slide it onto the surface of the media saturated pad.

27) Replace Petri dish cover.

28) Place Petri dishes into a 4.5 X 9 inch sterile sampling bag. Make sure not contaminate the inside of the bag.

29) Seal bag.

30) Place bag, with Petri dishes face down, into water bath at 44.5°C ± 0.2°C for 24 ± 2 hours.

31) Log initials, time, and date in the Microbiology bench sheet.

32) After 24 ± 2 hours, count blue colonies (See Section 10).

33) Log results, initials, time, and date in the Microbiology bench sheet.

8.2 Before and After Blanks

1) Blanks must be analyzed before and after filtration of a set of samples.

2) Before any sample is filtered, place a sterile filter in the filter holder unit.

3) Add 50 mL peptone buffer to filter unit.

4) Turn on vacuum and filter the buffer, this will be the Before Blank.

5) Remove and plate filter as indicated in Section 8.1.

6) Proceed with sample filtration and plating.

7) Run a Known Positive after all samples have been filtered (See Section 8.3).

8) Place a sterile filter in the filter holder unit.

9) Add 50 mL peptone buffer to filter unit.

10) Turn on vacuum and filter the buffer, this will be the After Blank.

11) Remove and plate filter as indicated in Section 8.1.

12) Log results, initials, time, and date in the Microbiology bench sheet.

8.3 Known Positive

1) After all samples have been filtered, a Known Positive is filtered and plated to ensure growth.

2) Place a sterile filter in the filter holder unit.

3) Add 10-20 mL peptone buffer to filter unit, then pipet 1.0 mL of mixed liquor (or suitable sample with known fecal coliforms) into filter unit.

4) Turn on vacuum and filter the sample, this will be the Known Positive.

5) Remove and plate filter as indicated in Section 8.1.

6) Log results, initials, time, and date in the Microbiology bench sheet.

8.4 Peptone Buffer Preparation

1) Measure 1.0 g Peptone powder into 1L volumetric flask.

2) Bring to volume with Nanopure water. Mix well.

3) Pour Peptone buffer into Nalgene® autoclavable squeeze bottle.

4) Take a sample of the buffer and measure pH, it should be 7.00 ± 0.1.

5) Loosely screw caps/dispensers onto squeeze bottles.

6) Autoclave Peptone buffer solutions.

7) Allow to cool, then transfer squeeze bottles to Micro fridge.

8.5 Helpful Hints

1) If the bacterial density of the sample is unknown, filter and plate out several volumes or dilutions in order to achieve a countable density. The volumes and/or dilutions should be expected to yield a countable membrane. In addition, select two additional quantities representing one-tenth and ten times this volume, respectively.

2) Separate filter holder units may be required during a set of samples. These will be indicated in the bench sheet.

3) Do not use damaged or bent membrane filters.

4) Rinse the filter unit thoroughly with Peptone buffer to avoid cross contamination.

9. QA/QC Requirements

9.1 Before and After Blanks must be run with each set of samples tested.

9.2 One duplicate per test series must be run.

9.3 One "Known positive" must be run per test series.

10. Expected Results

10.1 KPDES Permit Requirements
200 CFU/100 mL for Monthly geometric mean (GED)
400 CFU/100 mL for Maximum Weekly GED

In the event that the GED is exceeds the KPDES permit requirements, notify the Plant Superintendent and the Laboratory Supervisor.

10.2 Process Ranges
< 1 to >120,000 CFU/100 mL

11. Data Analysis and Calculations

11.1 See SOP – TB Fecal Coliforms for calculations.

12. Bibliography

12.1 U.S. EPA. Microbiological Methods for Monitoring the Environment: Water and Wastes. EPA 600/8-78-017. U.S. Environmental Protection Agency; Environmental Monitoring and Support Laboratory, Office of Research and Development, Washington, DC, 1978. Page 124.

12.2 Kentucky Department for Environmental Protection, Kentucky Division of Water and the Kentucky Division of Compliance Assistance. Discharge Monitoring Report Manual. 2009. August 10, 2009 revision. 28 pp.

WH Total Phosphorus Analysis
HACH Method 8190, PhosVer 3 with Acid Persulfate Digestion per EPA Method 365.2

1. Scope, Significance to Process and Application

Phosphorus in wastewater is almost always present in the form of phosphates. There are three major classes of phosphates; (1) Orthophosphates (fertilizer is major source), (2) Polyphosphates (detergents and cleaning agents are major sources), and (3) Organic Phosphates (biological waste is major source). Organic Phosphates are also formed from orthophosphates during biological treatment of waste streams. Analysis of Total Phosphorus includes all of the aforementioned forms of Phosphorus. The reduction of Total Phosphorus levels throughout the wastewater treatment process is highly important, as phosphorus concentrations in plant effluents must be low enough (See Sec.10.1, Permit Requirements) to avoid detrimental effects on the receiving environment, such as algae blooms.

2. Summary of Method

2.1 Executive Summary

Total phosphorus analysis at West Hickman Laboratory refers to the spectrophotometric analysis of all phosphorus forms in a water/wastewater sample that has been digested.

2.2 Discussion

West Hickman Lab uses, EPA approved, HACH Method 8190 with a HACH DR/4000 spectrophotometer. Method range is 0.0 to 1.1 mg/L as Total P and 0.0 to 3.5 mg/L as PO_4^{3-}, with an Estimated Detection Limit (EDL) of 0.06 mg/L PO_4^{3-}. The sample is first subjected to an acid persulfate digestion at 150˚C, reducing all phosphorus forms to reactive form, which reacts with molybdate reagent in an acid medium producing phosphomolybdate complex. This complex is reduced with ascorbic acid forming a molybdenum blue color with intensity proportional to the total phosphorus concentration, which is then quantifiable by the spectrophotometer.

3. Health & Safety Precautions

3.1 During sample digestion, the digester block is HOT (150˚C), Burn Hazard. Use digester block safety shields. In addition, the capped glass sample vials contain hot (150˚C) acidic solution under pressure.

3.2 PhosVer3 powder pillow can be a respiratory hazard. Wear a mask or place samples in hood when dispensing.

3.3 Wastewater samples should be considered potentially hazardous. Use proper personal protective equipment.

4. Sample Handling and Preservation

4.1 Analyze samples immediately after collection for best results. If prompt analysis is impossible, preserve samples for up to 28 days by adjusting the pH to 2 or less with H_2SO_4 and storing at 4˚C. Prior to analysis, allow samples to warm to room temperature and neutralize. Document sample preservation.

5. Reagents

5.1 PhosVer 3 Phosphorus Reagent Powder.
5.2 Potassium Persulfate Reagent Powder.
5.3 Sodium Hydroxide Solution 1.56 N.
5.4 Total and Acid Hydrolyzable Test Vials (Test'N Tubes).
5.5 Nanopure Grade Water.
5.6 Phosphate Standard Solution 1 mg/L as PO_4^{3-}.

6. Equipment & Lab Ware

6.1 HACH DBR200 COD Reactor with Test'N Tube block calibrated to 150˚C, with safety shields.
6.2 HACH DR/4000 Spectrophotometer with Test'N Tube adapter.
6.3 5 mL micropipetter with tips.
6.4 2 mL micropipetter with tips.
6.5 Microfunnel.

7. Interferences

7.1 Do not use commercial detergents containing phosphate for cleaning any lab ware utilized in this method.
7.2 Excess Turbidity.

8. Procedures

8.1 <u>Steps</u>

1) Turn the HACH DRB200 COD reactor ON and preheat to 150˚ C by pushing "Start".

2) Prepare a Reagent Blank by adding 5 mL of Nanopure lab water to a Total and Acid Hydrolyzable Test Vial and label it "Reagent Blank".

3) Prepare a Low Range Standard by adding 1 mL of phosphate standard solution (1 mg/L as PO_4^{3-}) and 4 mL of Nanopure lab water to a Total and

Acid Hydrolyzable Test Vial and label it "Low Standard" to produce a standard of 0.2 mg/L PO_4^{3-}.

4) Prepare a High Range Standard by adding 5 mL of phosphate standard solution (1 mg/L as PO_4^{3-}) to a Total and Acid Hydrolyzable Test Vial and label it "High Standard" to produce a standard of 1.0 mg/L PO_4^{3-}.

5) Put 5 mL of each sample to be analyzed in appropriately labeled Total and Acid Hydrolyzable Test Vials. Make sure to save samples until samples are read in case samples need to be diluted and/or reanalyzed.

6) Using a microfunnel, add to each test vial the contents of one potassium persulfate powder pillow, screw cap on tight, shake for 15 seconds, and place into the COD reactor. Set the reactor timer to run for 30 minutes.

7) CAREFULLY (vials are 150˚C) transfer hot vials to test tube racks and allow to cool to ambient temperature before proceeding. Note: Vials are under pressure until they cool (See Sec. 3.1).

8) To each vial add 2 mL of sodium hydroxide solution 1.56N, cap and shake to mix.

9) Turn the DR/4000 ON and allow the unit to go through its startup and self-check routine. Press the soft key under "HACH PROGRAM", type in the number 3036 and press "ENTER". The unit will respond by displaying "HACH PROGRAM 3036 P Total TNT" and request to be zeroed.

10) To the blank, standards, and samples add to each the contents of one PhosVer3 powder pillow, cap and shake for 15 seconds to mix, then allow a 2 minute reaction period before proceeding to step 11. Respiratory hazard (See Sec. 3.2). (Note: Step 11 must be completed within 6 minutes of the end of the 2 minute reaction period).

11) Place the reagent blank into the DR/4000 cell holder, close the lid and press "ZERO". Make sure the instrument is reading concentration and "FORM: P". One at a time, place all the vials (standards and samples) into the unit. Note and record the indicated values on the bench sheet. If desired, during the reading process, the soft arrow keys can be use to select units of P, PO_4^{3-}, or P_2O_5 although generally the "P" value (Total Phosphorus) will be recorded.

8.2 Helpful Hints

1) Analysis results are directly proportional to sample volumes; therefore it is very important that accurate sample volume measurements are made.

2) The Total and Acid Hydrolyzable Test Vials are cuvettes to be analyzed spectrophotometrically, and must provide a clear optical path. Prior to reading, clean the vials by wiping them down a Kim Wipe moistened with lab water, and then wipe them with a dry Kim Wipe.

3) When washing lab ware involved with this method, use only phosphate free cleaning agents, 1:1 HCL solution is recommended, followed by thorough Nanopure lab water rinse. Note: Most of the lab ware used in this method is disposable.

9. QA/QC Requirements

9.1 A Reagent Blank, High Standard (1.0 mg/L), and a Low Standard (0.2 mg/L) must be run with every analytical run.

9.2 A total of 5% of all samples must be run in duplicate.

9.3 Data acceptance criteria:

9.3.1 Analysis values for Standards must agree within 10% of the Standards known value or 0.12 mg/L, whichever is largest.

9.3.2 Duplicate values must agree within 5%.

If these criteria are not met, corrective action is indicated. See Quality Assurance Program (QAP) Sec. 15 “Corrective Action Policies and Procedures”.

10. Expected Results

10.1 KPDES Permit Requirements

1) West Hickman Wastewater Treatment Plant effluent limitations are:
   a. November 1st through April 30th - a monthly average of 1 mg/L and a weekly average of 2 mg/L.
   b. May 1st through October 31st - there are no effluent limitations on Total Phosphorus.

   Plant effluent composite samples are analyzed daily. Monthly and weekly averages are reported.

In the event that analysis results indicate values greater than the KPDES permit requirements, retest. If the value indicated by the retest is greater than KPDES

permit requirements, **Immediately** notify the Plant Superintendent and the Laboratory Supervisor.

10.2 Process Ranges

Typical phosphorus concentration values for plant influent vary primarily due to rainfall. Within the treatment process phosphorus concentrations can vary due to microbiological processes involving the release and uptake of phosphorus forms. Typical values for each plant are:

West Hickman Influent	2 mg/L to 12 mg/L
West Hickman Effluent	<1.0 mg/L

11. Data Analysis and Calculations

11.1 Concentration values are read directly from the DR/4000 spectrophotometer.

12. Bibliography

12.1 HACH DR/4000 Procedure Manual, EPA approved Method 8190 Phosphorus, Total. HACH Company, Loveland, CO.

12.2 Standard Methods Part 4500-P. Phosphorous. APHA-American Public Health Association *Standard Methods for the Examination of Water and Wastewater*; 21th edition ed.; American Water Works Association and Water Pollution Control Federation: Washington, DC, 2005.

12.3 U.S. EPA Method 365.2 Phosphorous, All Forms (Colorimetric, Ascorbic Acid, Single Reagent) Revised March 1983. Methods for Chemical Analysis of Water and Wastes. EPA-600-4-79-020. U.S. Environmental Protection Agency; Office of Research and Development, Washington, DC, 1982.

APPENDIX D LABORATORY BENCHSHEETS

TOWN BRANCH & WESTHICKMAN WASTEWATER TREATMENT PLANT

Town Branch Laboratory

Grab Samples

pH Method = EPA Method 150.1 pH (Electrometric) Issued 1971 (Editorial revision 1978 and 1982).

pH Meter Calibration

Temperature	= °C		Slope %		**Second Calibration**		Enter Date
First Buffer	= 7.00 pH		Calibrated By		First Buffer	= 7.00 pH	
Second Buffer	= 4.00 pH				Second Buffer	= 4.00 pH	
Third Buffer	= 10.00 pH				Third Buffer	= 10.00 pH	
					Calibrated By		

pH Enter Date

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Log Number															
Sample Name															
Date Samples Collected															
Time Analysis Started															
pH (Standard Units)															
Analyst															
Date Analysis Completed															

PTE ph

KPDES Permit Limits on Plant Effluent

6.0 mg/L is the Lowest pH we can have on any given sample.

9.0 mg/L is the Highest pH we can have on any given sample.

Time sampled:_____ Within 15 min limit: _____

Time received:_____

Time started:_____

Total Alkalinity, Orion Research Incorporated Laboratory Products Group Enter Date

	1	2	3	4	5	6	7	8	9
Log Number									
Sample Name	**Blank**	**Alkalinity Standard**							
Date Samples Collected									
Time Analysis Started									
Volume of Sample (mL)	10.0	10.0							
Dilution Factor = D	1.0	1.0							
pH After Addition of Reagent									
Reading from Chart = A									
Total Alkalinity = (A*D) mg/L as $CaCO_3$ - Blank									
Analyst									
Date Analysis Completed									

Volatile Acids Enter Date

	1	2	3
Log Number			
Sample Name			
Date Samples Collected			
Time Analysis Started			
V = Volume of Sample (mL)			
N = Normality of the NaOH			
A = Volume of NaOH Used			
F = Recovery Factor			
Volatile Acid (A*N*60,000)/(V*F)			
Analyst			
Date Analysis Completed			

Presevation: None required. Analyze Immediately.

Make Dilutions for all sample with Total Alkalinity > 225 mg/L $CaCO_3$

Total Alkalinity Control Limits are from 93 to 107 mg/L on the 100mg/L Standard (Standards pH should be 4.41 ± 0.05 after reagent addition)

TOWN BRANCH & WEST HICKMAN WASTEWATER TREATMENT PLANT

Town Branch Laboratory

Samples

Nitrogen, Ammonia = ULR TNT 830; HR TNT 832 /DR 5000

Nitrogen, Ammonia Date: Sunday, October 18, 2009

Time Analysis Started	Log Number	Date Sample Collected	Sample	Reading From Spec.	Sample Volume Use	Dilution	TNT
				mg/L	mL	v/v	
	23213	10/19/09	Low Standard 1.0 mg/L		5.0		ULR
	23213	10/19/09	High Standard 10.0 mg/L		0.2		
	23146	10/18/09	TB Raw Influent Sun		0.2		
	23149	10/18/09	TB Plant Effluent Sun		5.0		ULR
	23153	10/18/09	TB Creek Above Plant		5.0		ULR
	23192	10/18/09	WH Raw Influent Sun		0.2		
	23194	10/18/09	WH Plant Effluent Sun		5.0		ULR
	23137	10/17/09	TB Raw Influent Sat		0.2		
	23138	10/17/09	TB Plant Effluent Sat		5.0		ULR
	23185	10/17/09	WH Raw Influent Sat		0.2		
	23186	10/17/09	WH Plant Effluent Sat		5.0		ULR
	23217	10/19/09	Above Leak		5.0		ULR
	23218	10/19/09	Below Leak		5.0		ULR
	23219	10/19/09	In Spring		5.0		ULR

Analyst: _____

Quality Control Manager Is To Receive A Copy Of All "Q/C Results".

Presevation: Cool, 4^{O} C H_2SO_4 to pH < 2. Maximum Holding Time 28 days.

EPA Limit	Averages		EPA Limit	Averages	
	Monthly	Weekly		Monthly	Weekly
TB PTE In Winter.	7 mg/L	10.5 mg/L	WH PTE In Winter.	10 mg/L	15 mg/L
TB PTE In Summer.	2 mg/L	3 mg/L	WH PTE In Summer.	4 mg/L	6 mg/L

TOWN BRANCH WASTEWATER TREATMENT PLANT

Town Branch Laboratory

Carbonaceous Biochemical Oxygen Demand & Biochemical Oxygen Demand Method

EPA Method 405.1 (5-Days @ 20 ºC)

BOD_5 & $CBOD_5$

SEED CORRECTION DATA

Bottle Number			BL1	BL2	BL3
Volume of Seed Used in Seed Control	= S	(mL)	1	2	3
Initial D.O. of Seed Control Bottle	= B_1	(mg/L)			
5-Day D.O. of Seed Control Bottle	= B_2	(mg/L)			

Date Sample Collected: _____

Date Sample Collected if Different from the above date: _____

Date Incubation Started: _____ Date Incubation Stopped: _____

Unseeded Blank: Initial D.O.: _____ 5-Day D.O.: _____

Winkler Method: Initial D.O.: _____

Sodium Sulfite Added	Plant Effluent	

BIOCHEMICAL OXYGEN DEMAND CALCULATIONS

Bottle Number																		
Log Number																		
Sample Name																		
Date Sample Collected																		
Time Analysis Started																		
Was Nitrification Inhibitor Used (Y or N)																		
Sample Volume	= V	(mL)																
Initial D.O. of Sample	= D_1	(mg/L)																
5-Day D.O. of Sample	= D_2	(mg/L)																

B.O.D. QC Actual Value 198 mg/L

Analyst Setting Up Analysis: _____

Analyst Reading & Calculating Analysis: _____

D.O. = Dissolved Oxygen
B.O.D = Biochemical Oxygen Demand
$B.O.D._5$ = 5-Day Biochemical Oxygen Demand
C.B.O.D. = Carbonaceous Biochemical Oxygen Demand

KPDES Permit Limits on Plant Effluent

10 mg/L for Monthly Average

15 mg/L for Maximum Weekly Average

QUALITY CONTROL MANAGER IS TO RECEIVE A COPY OF ALL QC RESULTS.

Presevation: Cool, 4^{O}C. Maximum Holding Time 48 hours.

TOWN BRANCH WASTEWATER TREATMENT PLANT

Town Branch Laboratory

Grab Samples

Total Chlorine Residual Enter Date

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Analysis Method	EPA Method 330.1 Chlorine, Total Residual (Titrimetric, Amperometric) Issued 1978								EPA Method 330.5 Chlorine, Total Residual (Spectrophotometric, DPD) Issued 1978							
Log Number																
Sample Name																
Date Samples Collected																
Total Chlorine Residual (mg/L)																
Analyst																
Date Analysis Completed																
Time Analysis Completed																

PTE Chlorine Residual
Time sampled:_____ Within 15 min limit: _____
Time received:_____
Time started:_____

QUALITY CONTROL MANAGER IS TO RECEIVE A COPY OF ALL QC RESULTS.

Presevation: None required. Analyze Immediately.

KPDES Permit Limits on Plant Effluent

0.01 mg/L for Monthly Average

TOWN BRANCH WASTEWATER TREATMENT PLANT

Town Branch Laboratory

Grab Samples

Dissolved Oxygen Method = EPA Method 360.3 Oxygen, Dissolved (Luminescent Probe) Issued 2006.

Dissolved Oxygen Meter Calibration

Barometer Reading		Dissolved Oxygen from HQ40D			
Temperature, ºC:		Is Difference Less Than 0.2 Y or N			
Dissolved Oxygen Calibration Point		Calibrated By			

Dissolved Oxygen

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Log Number	23172	23173	23176												
Sample Name	**R**	***PTE***	**A Creek**												
Date Samples Collected	10/19/09	10/19/09	10/19/09												
Time Analysis Started															
Dissolved Oxygen Reading (mg/L)															
Analyst															
Date Analysis Completed	10/19/09	10/19/09	10/19/09												

QUALITY CONTROL MANAGER IS TO RECEIVE A COPY OF ALL QC RESULTS.

Presevation: None required. Analyze Immediately.

PTE Dissolved Oxygen
Time sampled:_____ Within 15 min limit: _____
Time received:_____
Time started:_____

KPDES Permit Limits on Plant Effluent

7.0 mg/L is the Lowest Dissolved Oxygen we can have on any given sample.

TOWN BRANCH & WEST HICKMAN WASTEWATER TREATMENT PLANT

Town Branch Laboratory

Total Coliforms Method: EPA 600/8-78-017 Microbiological Methods for Monitoring the Environment: Water and Wastes, page 108
Fecal Coliforms Method: EPA 600/8-78-017 Microbiological Methods for Monitoring the Environment: Water and Wastes, page 124
Fecal Streptococci Method: EPA 600/8-78-017 Microbiological Methods for Monitoring the Environment: Water and Wastes, page 136

Microbiology

	1	2	3	4	5		7	8	9	10	11	12	13	14	15
Log Number	**Blank**	23174	23174	23174	23177	Separate	23207	23207	23207	Known	**Blank**				
Sample Name	**Before**	**TB PTE**	**TB PTE**	**TB PTE**	**TB A Crk**	Filter	**WH PTE**	**WH PTE**	**WH PTE**	Positive	**After**				
Analysis	FC	FC	FC	FC	FC		FC	FC	FC	FC	FC				
Sample Volume = V (mL)	50	10	10	100			10	10	100		50				
Colonies Counted = A															
Colonies /100 mL = (A/V) x 100															
Averages															
Date Sample Collected	01/00/00	01/00/00	01/00/00	01/00/00	01/00/00		01/00/00	01/00/00	01/00/00	01/00/00	01/00/00				
Time Sample Collected															
Time Sample Received by Laboratory															
Date Analysis Started	01/00/00	01/00/00	01/00/00	01/00/00	01/00/00		01/00/00	01/00/00	01/00/00	01/01/00	01/00/00				
Time Analysis Started															
Set-up Analyst															
Date Analysis Completed	01/01/00	01/01/00	01/01/00	01/01/00	01/01/00		01/01/00	01/01/00	01/01/00	01/01/00	01/01/00				
Time Analysis Completed															
Reading Analyst															

Note: Plates should be read within 24hrs ± 2Hrs of set-up time.

Total Coliforms Method = TC
Fecal Coliforms Method = FC
Fecal Strep. Method = FS

QUALITY CONTROL MANAGER IS TO RECEIVE A COPY OF ALL QC RESULTS.

Presevation: Cool, 4^{O}C. Maximum Holding Time 6 hours.

KPDES Permit Limits on Plant Effluent
200/100mL for Monthly GED
400/100mL for Maximum Weekly GED

TOWN BRANCH & WEST HICKMAN WASTEWATER TREATMENT PLANT

Town Branch Laboratory

24 Hour Composite Samples

pH Method = EPA Method 150.1 pH (Electrometric) Issued 1971 (Editorial revision 1978 and 1982).

pH Meter Calibration **Second Calibration** Enter Date

Temperature	= °C		Slope %		First Buffer	= 7.00 pH	
First Buffer	= 7.00 pH		Calibrated By		Second Buffer	= 4.00 pH	
Second Buffer	= 4.00 pH				Third Buffer	= 10.00 pH	
Third Buffer	= 10.00 pH				Calibrated By		

pH Enter Date

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Log Number														
Sample Name														
Date Samples Collected														
Time Analysis Started														
pH (Standard Units)														
Analyst														
Date Analysis Completed														

KPDES Permit Limits on Plant Effluent

6.0 mg/L is the Lowest pH we can have on any given sample.

9.0 mg/L is the Highest pH we can have on any given sample.

Total Alkalinity, Orion Research Incorporated Laboratory Products Group

	1	2	3	4
Log Number				
Sample Name	**Blank**	**Alkalinity Standard**		
Date Samples Collected				
Time Analysis Started				
Volume of Sample (mL)	10.0	10.0		
Dilution Factor = D	1.0	1.0		
pH After Addition of Reagent				
Reading from Chart = A				
Total Alkalinity = (A*D) mg/L as $CaCO_3$				
Analyst				
Date Analysis Completed				

Total Hardness

	1	2	3
Log Number			
Sample Name			
Date Samples Collected			
Time Analysis Started			
Volume of Sample = V (mL)			
Molarity of the Titrant = M			
mL of Titrant Used = A			
Total Hardness = A mg/L as Total Hardness as $CaCO_3$			
Analyst			
Date Analysis Completed			

Presevation: None required. Analyze Immediately.

TOTAL PHOSPHORUS ANALYSIS, HACH TNT +, DR 5000 Date:

No.	Log Number	Date Sample Collected	Time Analysis Started	Sample	Pre-Dilution Factor	Sample Volume in mL	**Spec reading Total Phosphorus Conc. in mg/L as P**
1	23216			Standard Low (0.75 mg/L)	LR	2.0	
2	23216			Standard High (10.0 mg/L)	UHR	0.4	
3	23146			TB Raw Influent	UHR	0.4	
4	23146			TB Raw Influent Duplicate	UHR	0.4	
5	23148			TB Primary Effluent	UHR	0.4	
6	23149			TB Plant Effluent	LR	2.0	
7	23171			Mixed Liquor	LR	2.0	
8	23158			Return Activated Sludge	UHR	0.4	
9	23154			Raw Sludge Thickener Overflow	UHR	0.4	
10	23192			WH Raw Influent 1:10	LR	2.0	
11	23194			WH PTE	LR	2.0	
12	23137			TB Raw Influent Collected Sat	UHR	0.4	
13	23138			TB Plant Effluent Collected Sat	LR	2.0	

Analyst & Calibrated By: _____ Date of Analysis: Saturday, January 00, 1900

Interferences: Large amounts of turbidity may cause inconsistent results in the test because the acid present in the powder pillows may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles.
Aluminum > 200 mg/L, Arsenate, Chromium > 100 mg/L, Copper > 10 mg/L, Iron > 100 mg/L, Nickel > 300 mg/L extreme pH, Silica > 50 mg/L, Silicate > 10 mg/L, Sulfide > 90 mg/L, Zinc > 80 mg/L

TOWN BRANCH WASTEWATER TREATMENT PLANT

Town Branch Laboratory

24 Hour Composite Samples

Total Suspended Solids Method = Residue, Non-Filterable, EPA Method 160.2 (Gravimetric, Dried at 103 - 105 °C), Issued 1971.
Volatile Suspended Solids Method = Residue, Volatile, EPA Method 160.4 (Gravimetric, Ignition at 550 °C), Issued 1971.

Suspended Solids & Volatile Suspended Solids Enter Date

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Log Number														
Sample Name														
Additional Name Notations														
Date Sample Collected														
Time Analysis Started														
Tare Number														
Sample Volume = V (mL)														
Wt. Tare & Dried Solids = W_2 (gm)														
Wt. Tare = W_1 (gm)														
Wt. of Solids = $W_2 - W_1$ (gm)														
Total Suspended Solids $((W_2 - W_1)/V) \times 1{,}000{,}000$ = TSS (mg/L)														
Wt. Tare & Dried Solids = W_2 (gm)														
Wt. After 1 hr = W_3 (gm)														
Wt. Difference = $W_2 - W_3$ (gm)														
Is Difference < 0.0005 grams?														
Date Analyses Performed														

Analyst Setting Up Analysis: _____

Analyst Reading & Calculating Analysis: _____

KPDES Permit Limits on Plant Effluent
30 mg/L for Monthly Average
45 mg/L for Maximum Weekly Average
Presevation: None required. Maximum Holding Time 7 days.
QUALITY CONTROL MANAGER IS TO RECEIVE A COPY OF ALL QC RESULTS.

TOWN BRANCH & WEST HICKMAN WASTEWATER TREATMENT PLANT

Town Branch Laboratory

24 Hour Composite Samples

Total Solids Method = Residue, Total, EPA Method 160.3 (Gravimetric, Dried at 103 - 105 °C), Issued 1971.
Total Volatile Solids Method = Residue, Volatile, EPA Method 160.4 (Gravimetric, Ignition at 550 °C), Issued 1971.

Total Solids & Volatile Solids Enter Date

	1	2	3	4	5	6	7	8	9	10	11	12	13
Log Number													
Sample Name													
Additional Name Notations													
Date Sample Collected													
Time Analysis Started													
Tare Number													
Wt. Tare & Wet Sample = W_2 (gm)													
Wt. Tare = W_1 (gm)													
Wt. of Wet Sample = $W_2 - W_1$ (gm)													
Wt. Tare & Dried Solids = W_3 (gm)													
Wt. Tare = W_1 (gm)													
Wt. of Solids = $W_3 - W_1$ (gm)													
Total Solids $((W_3 - W_1)/(W_2 - W_1)) \times 1{,}000{,}000$ = TS (ppm)													
% Total Solids $((W_3 - W_1)/(W_2 - W_1)) \times 100$ = TS (%)													
Wt. Tare & Dried Solids = W_3 (gm)													
Wt. Tare & Ashed Solids = W_4 (gm)													
Wt. Volatilized Solids = $W_3 - W_4$ (gm)													
Volatile Solids $((W_3 - W_4)/(W_2 - W_1)) \times 1{,}000{,}000$ = VS (ppm)													
Volatile Solids $((W_3 - W_4)/(W_3 - W_1)) \times 100$ = VS (%)													
Date Analyses Performed													

QUALITY CONTROL MANAGER IS TO RECEIVE A COPY OF ALL QC RESULTS.

Analyst Setting Up Analysis: _____

Analyst Reading & Calculating Analysis: _____

TOWN BRANCH & WEST HICKMAN WASTEWATER TREATMENT PLANT

Town Branch Laboratory

Grab Samples

Total Suspended Solids Method = Residue, Non-Filterable, EPA Method 160.2 (Gravimetric, Dried at 103 - 105 °C), Issued 1971.

Volatile Suspended Solids Method = Residue, Volatile, EPA Method 160.4 (Gravimetric, Ignition at 550 °C), Issued 1971

Suspended Solids & Volatile Suspended Solids Enter Date

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Log Number															
Sample Name															
Additional Name Notations															
Date Sample Collected															
Time Analysis Started															
Tare Number															
Sample Volume = V (mL)															
Wt. Tare & Dried Solids = W_2 (gm)															
Wt. Tare = W_1 (gm)															
Wt. Tare & Ashed Solids = W_3 (gm)															
Date Analyses Performed															

QUALITY CONTROL MANAGER IS TO RECEIVE A COPY OF ALL QC RESULTS.

Presevation: None required. Maximum Holding Time 7 days.

Analyst Setting Up Analysis: _____

Analyst Reading & Calculating Analysis: _____

Log-book/Monday/Tbmonsht.xls/TB Suspended Solids, Grab

TOWN BRANCH & WEST HICKMAN WASTEWATER TREATMENT PLANT

Town Branch Laboratory

24 Hour Composite Samples

Total Solids Method = Residue, Total, EPA Method 160.3 (Gravimetric, Dried at 103 - 105 °C), Issued 1971.
Total Volatile Solids Method = Residue, Volatile, EPA Method 160.4 (Gravimetric, Ignition at 550 °C), Issued 1971

Total Solids & Volatile Solids Enter Date

	1	2	3	4	5	6	7	8	9	10	11	12	13
Log Number													
Sample Name													
Additional Name Notations													
Date Sample Collected													
Time Analysis Started													
Tare Number													
Wt. Tare & Wet Sample $= W_2$ (gm)													
Wt. Tare $= W_1$ (gm)													
Wt. Tare & Dried Solids $= W_3$ (gm)													
Wt. Tare & Ashed Solids $= W_4$ (gm)													
Date Analyses Performed													

QUALITY CONTROL MANAGER IS TO RECEIVE A COPY OF ALL QC RESULTS.

Analyst Setting Up Analysis: _____

Analyst Reading & Calculating Analysis: _____

Log-book/Monday/Tbmonsht.xls/TB Total Solids, 24Hr

TOWN BRANCH WASTEWATER TREATMENT PLANT

Town Branch Laboratory

Grab Samples

Total Solids Method = Residue, Total, EPA Method 160.3 (Gravimetric, Dried at 103 - 105 °C), Issued 1971.
Total Volatile Solids Method = Residue, Volatile, EPA Method 160.4 (Gravimetric, Ignition at 550 °C), Issued 1971

Total Solids & Volatile Solids Enter Date

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Log Number															
Sample Name															
Additional Name Notations															
Date Sample Collected															
Time Analysis Started															
Tare Number															
Wt. Tare & Wet Sample $= W_2$ (gm)															
Wt. Tare $= W_1$ (gm)															
Wt. Tare & Dried Solids $= W_3$ (gm)															
Wt. Tare & Ashed Solids $= W_4$ (gm)															
Date Analyses Performed															

QUALITY CONTROL MANAGER IS TO RECEIVE A COPY OF ALL QC RESULTS.

Analyst Setting Up Analysis: _____

Analyst Reading & Calculating Analysis: _____

Log-book/Monday/Tbmonsht.xls/TB Total Solids, Grab

TOWN BRANCH WASTEWATER TREATMENT PLANT

Town Branch Laboratory

24 Hour Composite Samples

Settleable Matter Method = EPA Method 160.5 Settleable Matter (Volumetric, Imhoff Cone) Issued 1974

Settleable Matter Enter Date

	1	2	3	4	5	6	7	8	9	10	11	12
Log Number												
Sample Name												
Date Samples Collected												
Vessel Used	Imhoff Cone	Imhoff Cone	Imhoff Cone									
Time Analysis Started												
Settleable Matter, 60 minutes = (mL/L/Hr.)												

Analyst: _____

Date Analysis Completed: _____

QUALITY CONTROL MANAGER IS TO RECEIVE A COPY OF ALL QC RESULTS.

Presevation: None required. Maximum Holding Time 48 hours.

TOWN BRANCH WASTEWATER TREATMENT PLANT

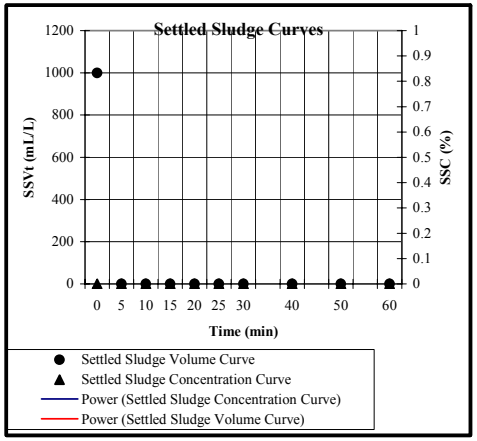
Town Branch Laboratory

Grab Samples

Settleable Matter

Enter Date

	1	
Log Number		
Sample Name	**Mixed Liquor**	
Date Samples Collected		
Vessel Used	Settlometer	
Settled Sludge Time = SST(min)	Settled Sludge Volume = SSV(mL/L)	Settled Sludge Concentration (SSC) = SSC_t = 1000 x (ATC/SSV_t)
Settleable Matter, 0 minutes = (mL/L)	1000	0.0%
5 minutes = (mL/L)		#DIV/0!
10 minutes = (mL/L)		#DIV/0!
15 minutes = (mL/L)		#DIV/0!
20 minutes = (mL/L)		#DIV/0!
25 minutes = (mL/L)		#DIV/0!
30 minutes = (mL/L)		#DIV/0!
40 minutes = (mL/L)		#DIV/0!
50 minutes = (mL/L)		#DIV/0!
60 minutes = (mL/L)		#DIV/0!
2 Hours = (mL/L)		#DIV/0!
3 Hours = (mL/L)		#DIV/0!
4 Hours = (mL/L)		#DIV/0!
Analyst		
Date Analysis Completed		



Rise Time	
Average Mixed Liquor Total Suspended Solids	
Average Mixed Liquor Volatile Suspended Solids	
Sludge Volume Index (SVI) = (SSV30 x 1000)/MLSS	
Sludge Density Index (SDI) = 100/SVI	

Centrifuge Spin Test	
Sludge Volume after 15-minute Centrifuge Spin (mL)	
Aeration Tank Mixed Liquor Concentration (ATC)	
Analyst	
Date Analysis Completed	

Activated Sludge Settled Volume Method = Method 2510 C. Settled Volume, WEF Standard Methods, 21st Edition.

Activated Sludge Zone Settling Rate Method = Method 2710 E. Zone Settling Rate, WEF Standard Methods, 21st Edition.

Sludge Volume Index Method = Method 2710D. Sludge Volume Index, WEF Standard Methods, 21st Edition.

Sludge Density Index Method = Method 2710D. Sludge Density Index, WEF Standard Methods, 21st Edition.

Centrifuge Spin Test = Centrifuge Method for Estimating Suspended Matter, WEF Simplified Laboratory Procedures for Wastewater Examination, 3rd Edition, 1985, Pg 30.

QUALITY CONTROL MANAGER IS TO RECEIVE A COPY OF ALL QC RESULTS.

Presevation: None required. Maximum Holding Time 48 hours.

Sunday's Sheets

Town Branch Laboratory

pH Meter Calibration 01/00/00

Temperature	=	°C			
First Buffer	=	7.00 pH			
Second Buffer	=	4.00 pH			
Third Buffer	=	10.00 pH			
Slope					
Calibrated By					
Log Number			23127	23128	
Sample Name			**PTE**	**M/L**	**STD**
Date Samples Collected			01/00/00	01/00/00	01/00/00
Time Analysis Started					
pH (Standard Units)					
Analyst					
Date Analysis Completed			01/00/00	01/00/00	01/00/00

Presevation: None required. Analyze Immediately.
KPDES Permit Limits on Plant Effluent pH
6.0 mg/L is the Lowest pH we can have on any given sample.
9.0 mg/L is the Highest pH we can have on any given sample.

pH Method = EPA Method 150.1 pH (Electrometric) Issued 1971 (Editorial revision 1978 and 1982).

Fill in yellow squares.

Charlie Begley 234 - 4886
Mark Stager 368 - 7296

Dissolved Oxygen Meter Calibration 04/26/63

Barometer Reading	
Temperature, ºC:	
Calculated Dissolved Oxygen Value	
Calibrated By	
Log Number	0000
Sample Name	**PTE**
Date Samples Collected	04/26/63
Time Analysis Started	
Dissolved Oxygen Reading, mg/L	
Analyst	
Date Analysis Completed	04/26/63

KPDES Permit Limits on Plant Effluent DO
7.0 mg/L is the Lowest Dissolved Oxygen we can have on any given sample.

Dissolved Oxygen Method = EPA Method 360.1 Oxygen, Dissolved (Membrane Electrode) Issued 1971

Fill in yellow squares.

Charlie Begley 234 - 4886
Mark Stager 368 - 7296

Sunday's Sheets

Microbiology

Log Number		23129	14969	
Sample Name	**Blank Before**	**PTE**	**PTE**	**Blank After**
Analysis	Fecal Coliform			
Sample Volume = V (mL)	50	10	100	50
Colonies Counted = A				
Colonies /100 mL = (A/V x 100)				
Averages:				
Date Sample Collected	Saturday, January 00, 1900			
Time Sample Collected				
Time Analysis Started				
Set-up Analyst				
Date Analysis Completed	Sunday, January 01, 1900			
Time Analysis Completed				
Reading Analyst				

Preservation: Cool, 4^{O}C. Maximum Holding Time 6 hours.
Note: Plates should be read within 24hrs ± 2Hrs of set-up time.

KPDES Permit Limits on Plant Effluent Fecal Coliform
200/100mL for Monthly GED 400/100mL for Maximum Weekly GED

Total Chlorine Residual 04/26/63

Analysis Method	EPA Method 330.1 Chlorine, Total Residual (Titrimetric, Amperometric) Issued 1978
Log Number	0000
Sample Name	**PTE**
Date Samples Collected	04/26/63
Time Analysis Started	
Total Chlorine Residual (mg/L)	
Analyst	
Date Analysis Completed	04/26/63
Time Analysis Completed	

B.D.L. = Below Detection Limit = < 0.01 mg/L

KPDES Permit Limits on Plant Effluent Res. Cl_2
0.010 mg/L for Monthly Average
0.019 mg/L for Daily Maximum

Presevation: None required. Analyze Immediately.

OAKTON® Waterproof TDSTestr and ECTestr Series Instructions

Before you Begin

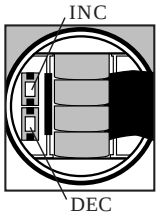
Remove electrode cap. Soak electrodes for a few minutes in alcohol to remove oils.

Calibration

Tester is factory calibrated. However, to ensure accuracy, calibrate on a regular basis.

Select a calibration standard appropriate for your Testr:
TDSTestr Low: from 200 to 1990 ppm
TDSTestr High: from 2.00 to 10.00 ppt
ECTestr Low: from 200 to 1990 µS
ECTestr High: from 1.00 to 19.90 mS

It is best to select a standard close to the test solution value.



1. Open battery compartment lid (end with lanyard loop). The two white buttons are Increment (INC) and Decrement (DEC) calibration keys.
2. Rinse electrode in deionized water, then rinse it in calibration standard, then dip it into a container of calibration standard.
3. Switch unit on (ON / OFF key). Wait several minutes for display to stabilize.
4. Press the INC or DEC keys to adjust reading to match the calibration standard value.
5. After 3 seconds without a key press, the display flashes 3 times, then shows "ENT". The tester accepts calibration value; returns to measurement mode.
6. Replace battery cap.

TDS or Conductivity Testing

1. Remove electrode cap. Switch unit on (ON / OFF key).
2. Dip electrode into test solution. Make sure sensor is fully covered.
3. Wait for reading to stabilize (Automatic Temperature Compensation corrects for temperature changes). Note reading.
4. Press ON / OFF to turn off Tester. Replace electrode cap. Note: Tester automatically shuts off after 8.5 minutes of nonuse.

HOLD function

Press HOLD key to freeze display. Press HOLD again to release.

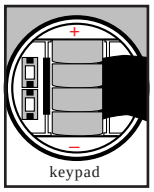
Setting TDS Factor (TDSTestrs only)

The TDSTestrs let you select a TDS factor of 0.4 to 1.0.

1. Open battery compartment. With meter on, press the HOLD key, then press the INC key (INC key is inside battery compartment; see diagram at left).
2. Press the INC or DEC keys to adjust the TDS factor.
3. After 3 seconds without a key press, the display flashes 3 times, then shows "ENT". Tester accepts TDS factor and returns to measurement mode.
4. Replace battery cap.

Changing Batteries

1. Open battery compartment lid (end with lanyard loop).
2. Remove old batteries; replace with fresh ones. Note polarity (shown in battery compartment and in picture at right).
3. Recalibrate after battery change.



Tester Maintenance

- To improve performance, clean the electrodes by rinsing them in alcohol for 10-15 minutes. Remove white plastic cup insert to clean viscous solutions.
- Replace all batteries if low battery indicator appears, or if readings are faint or unstable.
- If you experience drift, periodically let electrode fully dry.

When you need a new electrode, see "**Electrode Replacement"** at right.

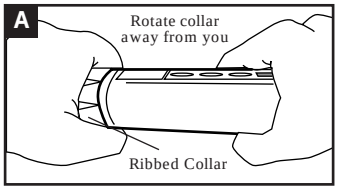
Specifications

Testr	TDS Low	TDS Hi	EC Low	EC Hi
Range	0 to 1990 ppm	0 to 10.00 ppt	0 to 1990 µS	0 to 19.90 mS
Resolution	10 ppm	0.10 ppt	10 µS	0.10 mS
Accuracy	±1% full scale			
TDS Factor	0.4 to 1.0 selectable		—	
Calibration Standard Range	200 to 1990 ppm	2.00 to 10.00 ppt	200 to 1990 µS	1.00 to 19.90 mS
Calibration	1 point (calibration range is ±30% of factory default parameter)			
ATC	0 to 50°C (2% per °C)			
Operating Temp.	0 to 50ºC			
Power	Four 1.5V alkaline batteries (Eveready A76BP; supplied) 150 hrs. continuous use Alternate replacement Model Eveready 303 silver oxide, 70 hrs. continuous use.			
Dimensions	6.5"L x 1.5" dia. (165 x 38mm)			
Weight	3.25 oz (90 gms)			

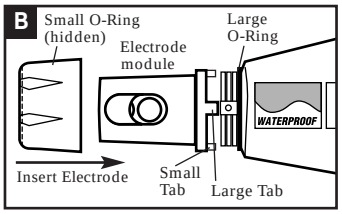
Electrode replacement:

You can replace the electrode module at the fraction of the cost of a new Testr. When the Testr fails to calibrate, gives fluctuating readings in buffers, shows error messages 'E2' or 'OR' in a buffer, and the procedures in the Maintenance section do not help, you need to change the electrode.

1. With dry hands, grip the ribbed Testr collar with electrode facing you. Twist the collar counter clockwise. *(see diagram A)*. Save the ribbed Testr collar and O-ring for later use.



2. Pull the old electrode module away from the Testr.
3. Align the four tabs on the new module so they match the four slots on the testr. *(see diagram B)*.



4. Gently push the module onto the slots to seat it in position. Push the smaller O-ring fully onto the new electrode module. Push the collar over the module and thread it into place by firmly twisting clockwise.

Warranty:

Each TDSTestr and ECTestr meter body is warranted against defects in materials and workmanship for a period of 12 months from the date of purchase; the electrode module is warrantied for a period of 6 months from the date of purchase. If repair, adjustment or replacement is necessary and has not been the result of abuse or misuse within the 6 month period, please return the Testr—freight pre-paid—and correction will be made without charge. Out of warranty products will be repaired on a charge basis.

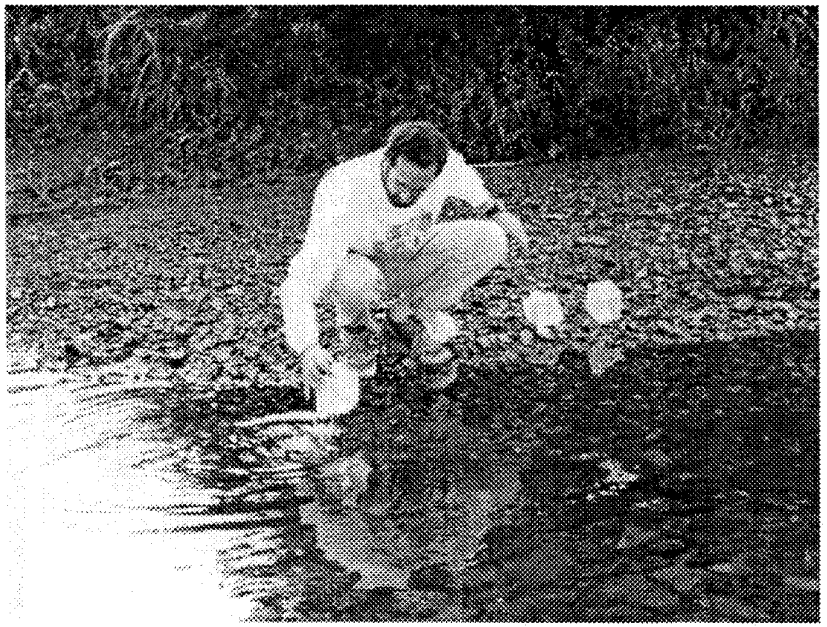
Return of Items:

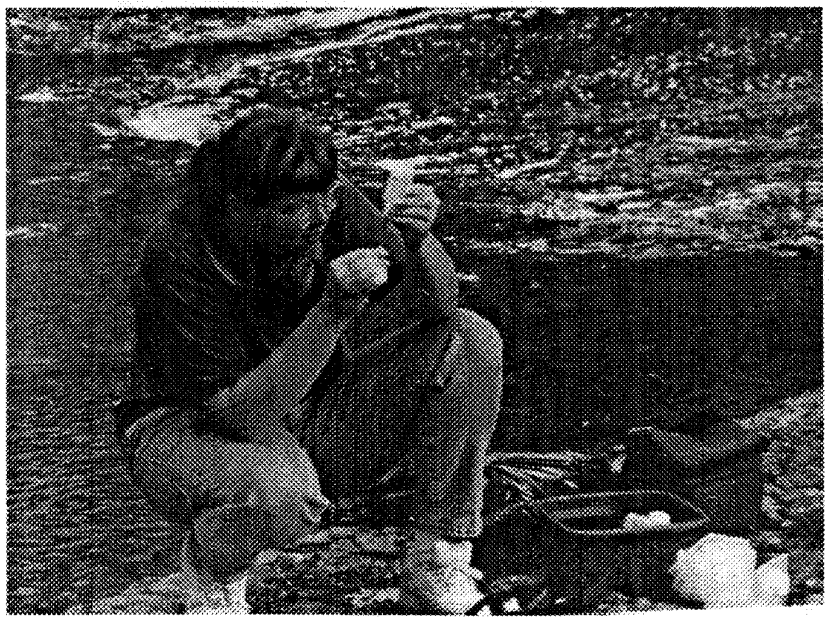
Authorization must be obtained from your OAKTON Distributor before returning items for any reason. When applying for authorization, please include information regarding the reason the item(s) are to be returned.

Note: We reserve the right to make improvements in design, construction and appearance of products without notice. Prices are subject to change without notice.

Watershed Watch Water Chemistry Sampling Methods

For
Field Chemistry and
Lab Analysis





pH

No chemical test is of greater or more universal significance than the measurement of pH. The pH value affects virtually all chemical and biological processes occurring in a solution.

pH is a measure of the acidic or basic (alkaline) nature of a solution. The concentration of the hydrogen ion [H+] activity in a solution determines the pH. Mathematically this is expressed as: **pH = - log [H+]**

Environmental Impact: A pH range of 6.0 to 9.0 appears to provide protection for the life of freshwater fish and bottom dwelling invertebrates

The most significant environmental impact of pH involves synergistic effects. **Synergy** involves the combination of two or more substances which produce effects greater than their sum.

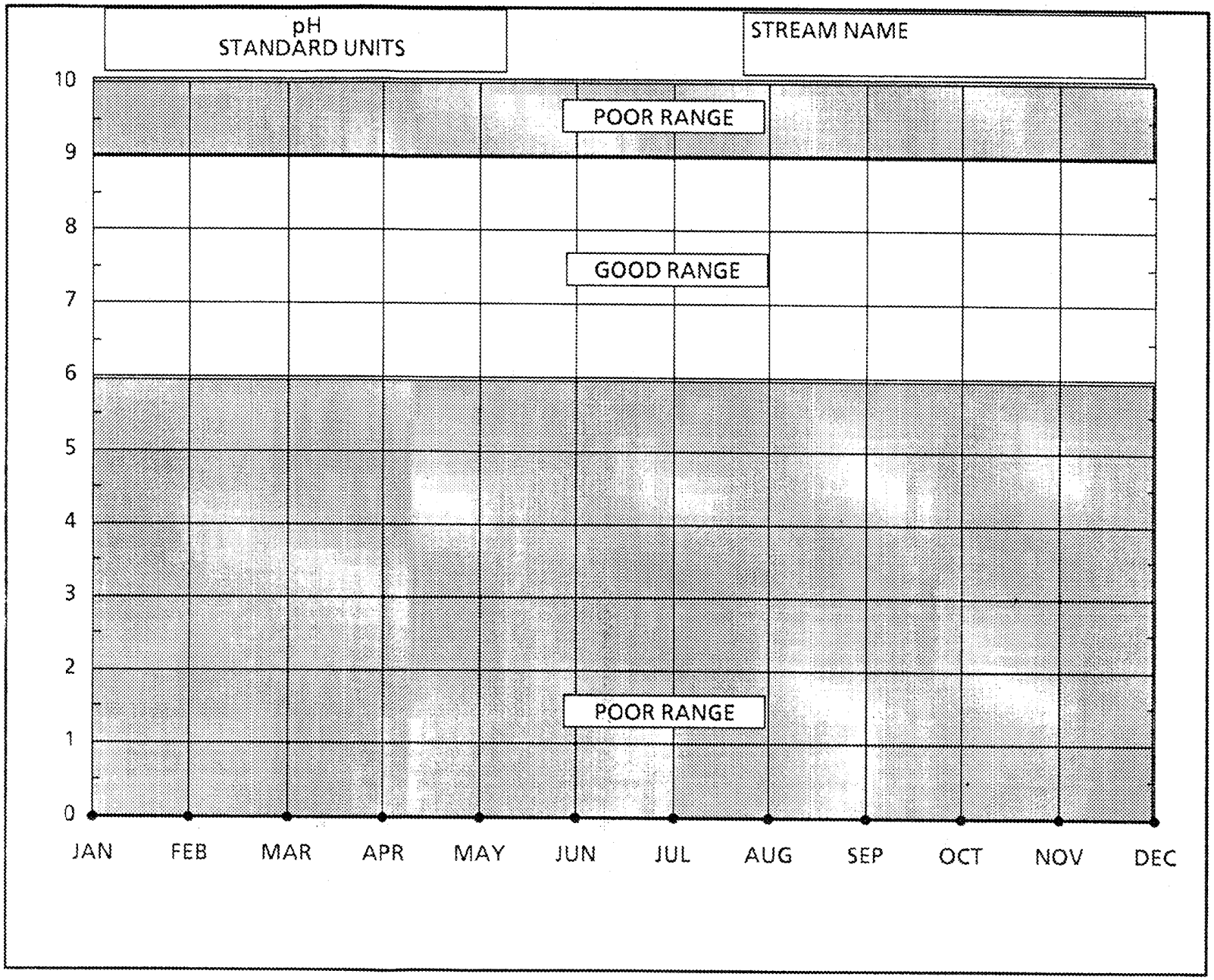
This process is important in surface waters. Runoff from agricultural, domestic and industrial areas may contain iron, aluminum, ammonia, mercury or other elements. The pH of the water will determine the toxic effects, if any, of these substances. For example, 4 mg/l of iron would not present a toxic effect at a pH of 4.8. However, as little as 0.9 mg/l of iron at a pH of 5.5 can cause fish to die.

Synergy has special significance when considering water and wastewater treatment. The steps involved in water and wastewater treatment require specific pH levels. In order for coagulation (a treatment process) to occur, pH and alkalinity must fall within a limited range. Chlorination, a disinfecting process for drinking water, requires a pH range that is temperature dependent.

The table below gives some special effects of pH on fish and aquatic life.

Limiting pH Values

Minimum	Maximum	Effects
3.8	10.0	Fish eggs could be hatched, but deformed young are often produced
4.0	10.1	Limits for the most resistant fish species
4.1	9.5	Range tolerated by trout
4.3	- - -	Carp die in five days
4.5	9.0	Trout eggs and larvae develop normally
4.6	9.5	Limits for perch
5.0	- - -	Limits for stickleback fish
5.0	9.0	Tolerable range for most fish
- - -	8.7	Upper limit for good fishing waters
5.4	11.4	Fish avoid waters beyond these limits
6.0	7.2	Optimum (best) range for fish eggs
1.0	- - -	Mosquito larvae are destroyed at this pH value
3.3	4.7	Mosquito larvae live within this range
7.5	8.4	Best range for the growth of algae


pH
STANDARD UNITS
STREAM NAME
10
9
8
7
6
5
4
3
2
1
0
POOR RANGE
GOOD RANGE
POOR RANGE
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC

pH Test Procedure

1. Rinse the test tube with the water to be tested, then refill the tube to the 5 mL line. Tube 0230
2. Add the indicator solution to the sample in the tube. Read the instruction label on the front of the comparator to determine the proper number of drops of indicator solution to be added. Hold the dropper bottle or pipet vertically (not tilted) to dispense uniformly-sized drops. – 10 Drops – Wide Range Indicator
3. Cap the tube and invert several times to mix the contents.
4. Insert tube in comparator and match the color of the test sample against the color standards to obtain the pH test result.

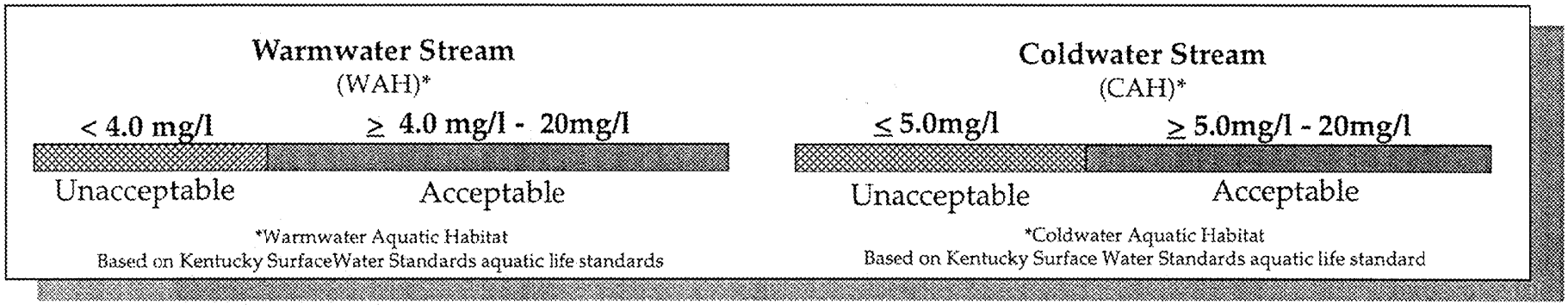
Test Equipment Care & Maintenance

1. Carefully wash and rinse test tubes after each use.
2. Tighten the reagent container cap immediately after use.
3. Avoid prolonged exposure of all test components to direct sunlight.
4. Avoid extreme high temperatures and protect all components from freezing.
5. Keep reagents and equipment out of reach of young children.
6. Anticipate your requirements for replacement reagents.

LaMOTTE CHEMICAL PRODUCTS COMPANY
PO BOX 329 • CHESTERTOWN • MARYLAND • 21620 (301) 778-3100

Dissolved Oxygen

Dissolved Oxygen is a critical factor in natural waters. Oxygen levels indictate the ability of a surface water to support a healthy environment of aquatic plants and animals. Low oxygen levels may signal contamination from organic wastes.



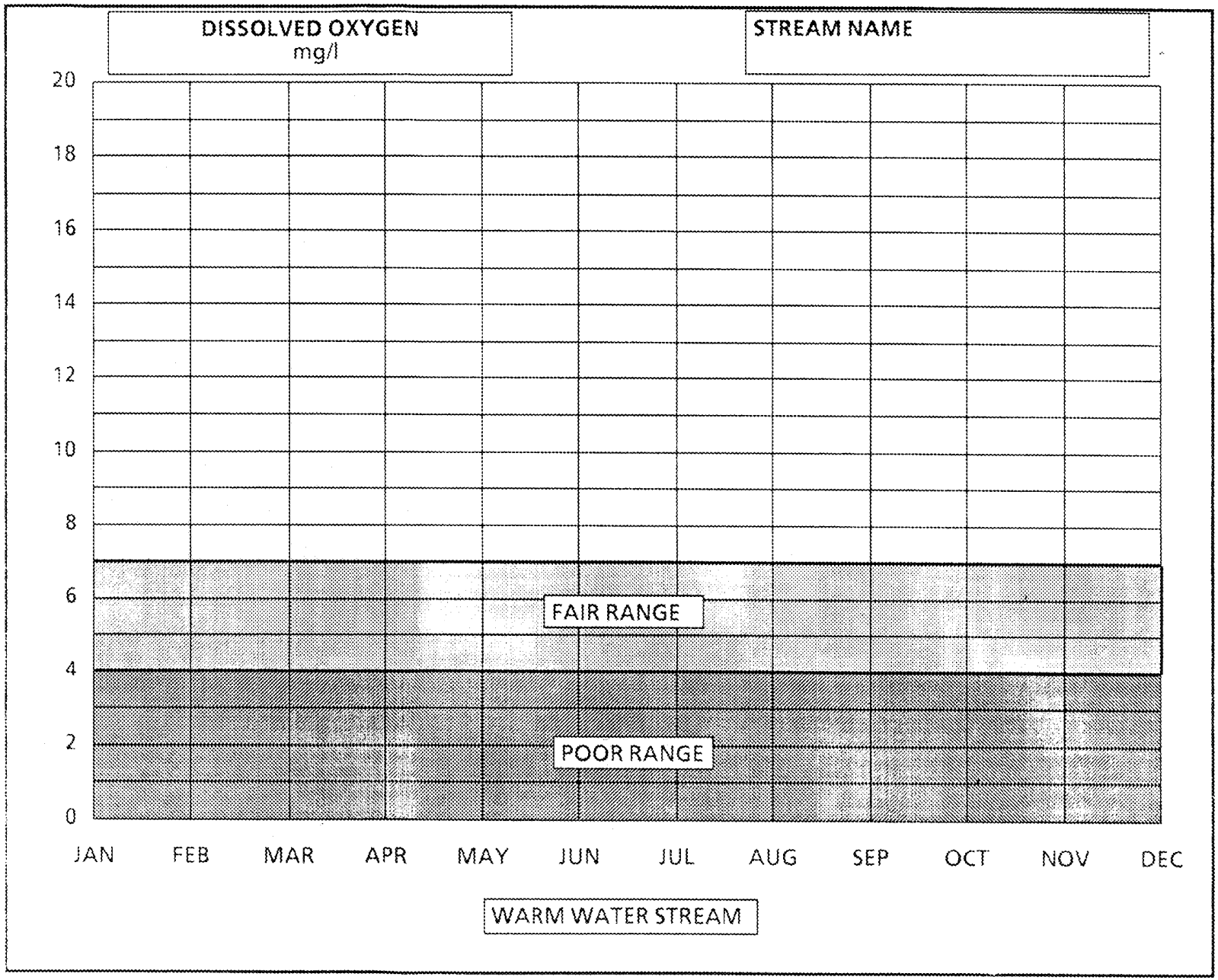
Dissolved oxygen (DO) analysis measures the amount of gaseous oxygen (O_2) dissolved in an aqueous solution. Oxygen gets into water by diffusion from the surrounding air, by aeration (rapid movement) and as a waste product of photosynthesis of aquatic plants, algae and phytoplankton.

Dissolved oxygen levels in water are dictated by the temperature, salinity of water and atmospheric pressure. Because of these factors, the DO level can be low even in highly aerated water.

Environmental Impact: Adequate dissolved oxygen is necessary for good water quality. Oxygen is a necessary element to all forms of life. Natural stream purification processes require adequate oxygen levels in order to provide for aerobic life forms. As dissolved oxygen levels in water drop below 5.0 mg/l, aquatic life is put under stress. The lower the concentration the greater the stress. Oxygen levels that remain below 1-2 mg/l for a few hours can result in large fish kills.

Organic material, such as found in wastewater, uses oxygen for decomposition. The amount of oxygen to decompose waste is called biochemical oxygen demand (BOD). When the BOD of the waste exceeds the available oxygen, the DO in the stream is reduced or depleted and is unavailable for fish and invertebrates.

Total dissolved gas concentrations in water should not exceed 110 percent. Concentrations above this level can be harmful to aquatic life. Fish in waters containing excessive dissolved gases may suffer from "gas bubble disease"; however, this is a very rare occurrence. The bubbles or emboli block the flow of blood through blood vessels causing death. External bubbles (emphysema) can also occur and be seen on fins, on skin and on other tissue. Aquatic invertebrates are also affected by gas bubble disease but at levels higher than those lethal to fish.


DISSOLVED OXYGEN
mg/l
STREAM NAME
20
18
16
14
12
10
8
6
4
2
0
FAIR RANGE
POOR RANGE
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
WARM WATER STREAM

DISSOLVED OXYGEN TEST KIT

LaMotte Chemical

Model EDO • Model AG-30 Code 7414

For determining the dissolved oxygen content of water, this test kit uses the azide modification of the Winkler Method and employs a LaMotte Direct Reading Titrator in the final titration.

Quantity	Contents	Code
25 mL	*Manganous Sulfate Solution	*4167-G
25 mL	*Alkaline Potassium Iodide Azide Solution	*7166-G
50 g	Sulfamic Acid, Powder	6286-H
50 mL	Sodium Thiosulfate Solution (0.025N)	4169-H
25 mL	Starch Indicator	4170-G
1	Direct Reading Titrator, 0-10	0377
1	Titration Tube, 20 mL	0299
1	Pipet, glass, with screw cap	0341
1	Bottle, Water Sampling, 60 mL, glass	0688-DO
1	Spoon, Measuring, 1.0 g	0697

WARNING: *Reagents marked with a * are considered hazardous substances. Material Safety Data Sheets (MSDS) are supplied for these reagents. For your safety read label and accompanying MSDS before using.

Carefully read the instruction manual for the LaMotte Direct Reading Titrator before performing the test procedure. The Titrator is calibrated in parts per million (ppm) Dissolved Oxygen. Each minor division on the Titrator scale equals 0.2 ppm.

Collection & Treatment Of The Water Sample

Steps 1 through 4 below describe proper sampling technique in shallow water. For collection of samples at depths beyond arm's reach, special water sampling apparatus is required. (e.g., the LaMotte Water Sampling Chamber, Code 1060; Model JT-1 Water Samplers, Code 1077; or Water Sampling Outfit, Code 3103).

1. To avoid contamination, thoroughly rinse the Water Sampling Bottle (0688-DO) with the water to be sampled.
2. Tightly cap the mouth of the bottle, submerge the bottle to the desired depth, and remove the cap to allow the bottle to fill.
3. Tap the sides of the submerged bottle to dislodge any air bubbles clinging to the inside of the bottle. Replace the cap while the bottle is still submerged.
4. Retrieve the bottle and examine it carefully to make sure that no air bubbles are trapped inside. Once a satisfactory sample has been collected, proceed immediately with Steps 5 & 6 to "fix" the sample.

NOTE: Be careful not to introduce air into the sample while adding the reagents in steps 5 & 6. Simply drop the reagents onto the test sample, cap carefully, and mix gently.

5. Add 8 drops of *Manganous Sulfate Solution (4167) and 8 drops of *Alkaline Potassium Iodide Azide Solution (7166) to the sample. Cap the bottle and mix by inverting several times. A precipitate will form. Allow the precipitate to settle below the shoulder of the bottle before proceeding.
6. Using the 1 g measuring spoon (0697), add one level measure of Sulfamic Acid Powder (6286) to the sampling bottle. Cap the bottle and gently shake to mix, until the reagent and the precipitate have dissolved. A clear-yellow to brown-orange color will develop, depending on the oxygen content of the sample. Following the completion of Step 6, contact between the water sample and the atmosphere will not affect the test result. Once the sample has been "fixed" in this manner, it is not necessary to perform the actual test procedure immediately. Thus, several samples can be collected and "fixed" in the field, and then carried back to a testing station or laboratory where the test procedure is to be performed.

Test Procedure

1. Fill the titration tube (0299) to the 20 mL line with the "fixed" sample and cap the tube. NOTE: If the color of the "fixed" sample is already a very faint yellow, skip Step 2, perform Step 3, and begin the titration at Step 4.
2. Fill the Direct Reading Titrator (0377) with Sodium Thiosulfate Solution (4169) in the manner described in the Titration manual. Insert the Titrator into the center hole of the titration tube cap. While gently shaking the tube, slowly press the plunger to titrate until the yellow-brown color is reduced to a very faint yellow.
3. Remove the titrator and cap. Be careful not to disturb the Titrator plunger, as the titration begun in Step 2 will be continued in Step 4. Using the pipet (0341), add 8 drops of Starch Indicator (4170). The sample should turn blue.
4. Replace the cap and Titrator and continue titrating until the blue color just disappears. Where the plunger tip meets the Titrator scale, read the test result in ppm Dissolved Oxygen.
5. If the plunger tip reaches the bottom line on the Titrator scale (10 ppm) before the endpoint color change occurs, refill the Titrator and continue the titration. When recording the test result, be sure to include the value of the original amount of reagent dispensed (10 ppm).

Order Reagent Refill Package By Code Number R-7414

LaMotte Chemical Products Company
PO Box 329 • Chestertown • Maryland • 21620
301-778-3100

Flow

What is it?

Flow is the **volume** of water a stream or river discharges over a given amount of time.

Flow is measured in cubic feet per second (cfs) or million gallons per day (mgd) By using the immediate flow rate for a water course the amount of material carried in the water can also be calculated.

Velocity is the distance that water travels over a given period of time; i.e. the **speed** of the water. Velocity is measured in feet per second (fps).

How does it behave in the aquatic environment?

Natural flows of streams will vary during the year and also from year to year. Animals and plants in and near streams depend on the natural flow patterns for growth, reproduction and the replenishment of nutrients in the land. For example, the river gum needs regular yearly flooding to survive, fish migrations need increased flow for spawning, and flood plains get their nutrient-rich soils from floods.

Any changes in flow may impact on ecosystems by interrupting the flow requirements of the animals and plants. The natural flow may enable the stream to absorb inputs from the surrounding watershed and so reduce adverse side effects.

Often the natural flow is changed by humans as a result of dam constructions to service demands by irrigation, livestock watering, industrial and urban purposes. A reduction in the natural flow will reduce the ability of the stream to absorb inputs. Alternatively, stream flow may be increased, enabling the storage of water during high flow events and the releasing of this stored water during drier times when there is high water demand by the community.

Flow and water quality

The water quality in a stream can vary considerably depending on flow.

- **Moderate to moderately-high flows**

  The highest water quality in a stream normally occurs under moderately-high to moderate flows. Under these conditions there is sufficient flow to ensure good oxygenation of the water, to dilute and flush out pollutants, and to limit the build-up of algae.

- **Low flows**

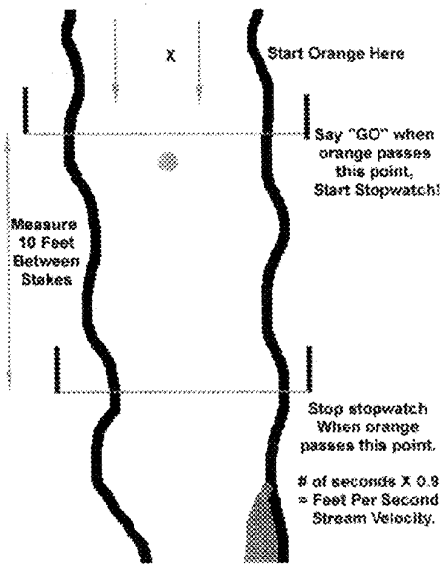
  Under low flow conditions, sections of a stream may become semi-stagnant resulting in low oxygen levels and, and nutrients may result in increased growth of algae. Salinity of the water may also increase and streams may be subject to larger variations in temperature which increases stress on the biota.

- **Flood flows**

  Under moderate and low flows, the water entering a stream is very largely derived from below-ground seepage. In contrast, during and immediately after heavy rainfall, water starts to flow over the surface of the ground. This water picks up all sorts of pollutants as it moves over the land surface and its quality is quite different to that of normal seepage inflow. As would be expected, such overland inflows cause marked changes to the normal quality of water in a stream. One obvious manifestation is the increase in suspended particles (i.e. the stream gets muddier) but there are changes to many other aspects of water quality. These changes in quality are generally greatest during the early stages of a flood, but may persist for days or even weeks after such an event.

  Because flow has a significant impact on water quality, it is important that we record it at the time of sampling (and if possible, during the previous few days). In particular we need to know if flows were 'normal', very low or very high.

Measuring stream velocity - easy


X
Start Orange Here
Say "GO" when orange passes this point, Start Stopwatch!
Measure 10 Feet Between Stakes
Stop stopwatch When orange passes this point.
# of seconds X 0.9 = Feet Per Second Stream Velocity.

1. Place a mark 10 feet upstream of your sampling site. Ideally, the stream bed should be straight, smooth and free of vegetation or other obstacles. If the flow is very slow, or if the size of your stream varies a lot over 10 feet, then use a shorter distance.
2. Position a person at each end of this length.
3. Drop an orange into the water 5 feet above your starting point to give it time to 'get into the flow'.
4. The first person calls 'Go' as the orange reaches the starting point and the second one starts the stopwatch. They stop the stopwatch when the orange reaches the end of the measured length.
5. Repeat measurement at three different locations across the stream, and average the results to give velocity in fps. An estimate of velocity is an essential measurement for calculating how much material the water is carrying.
6. Here is a catch. You have to divide your three readings by five to get your average velocity. The reason is the water next to each bank is not moving, so it has to be factored in.

$$\text{Corrected velocity} = \frac{\text{distance traveled (feet)}\_\_\_\_\_}{(\text{Time1}\_\_\_\_\_\ \text{Time2}\_\_\_\_\_\ \text{time3}\_\_\_\_\_)/5\ \text{(seconds)}}$$

Measuring stream flow - more difficult

The formula for measuring the flow of the stream is:

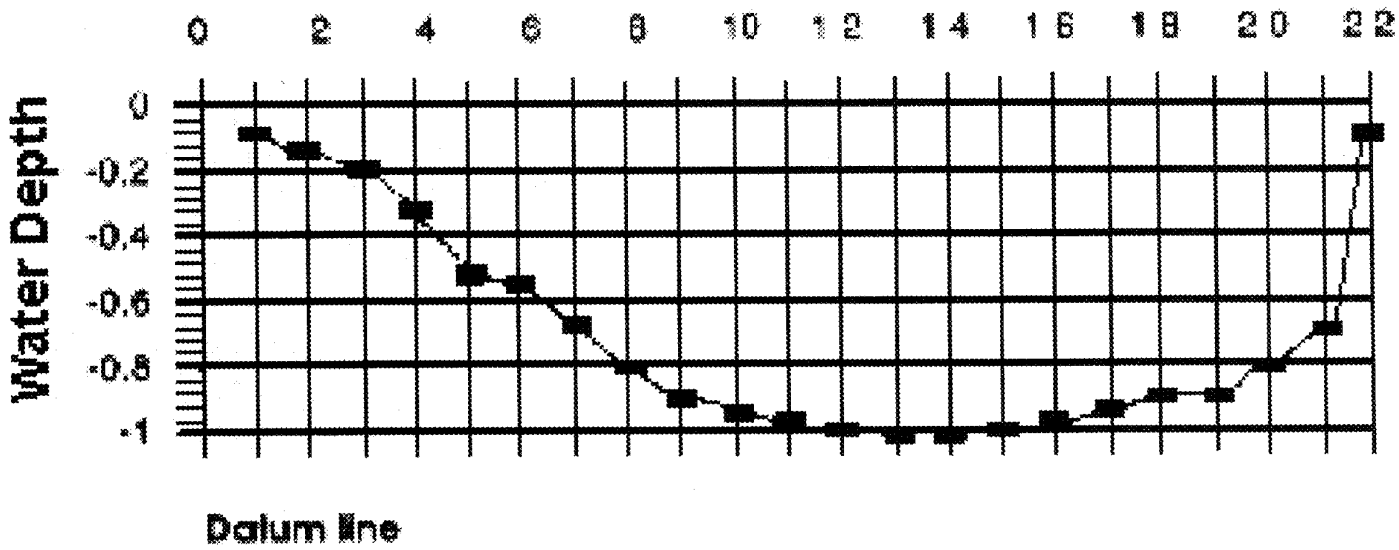
Stream flow (CFAs) = cross-sectional area of water (sq. feet) x velocity (fps)

For small streambeds that dry up for part of the year you should take these measurements when the streambed is dry. For permanently flowing streams find a site as close to your monitoring site as possible. Find a landmark such as a bridge or tree to mark the horizontal datum line and with depth measurements to give you a permanent measuring stick.

1. Use two posts or similar to stretch your measuring string (marked at an appropriate interval for your stream site) across the steam bed. (two foot intervals will do
2. pole from the bridge until it touches the stream bottom. Then measure the wet length of your pole to get the depth of the stream at that point. Repeat at each.
3. Record your measurements like this:

Points on datum line (feet)	Depth of Water
2	1
4	1.8
6	2

4. From your recorded measurements draw the drain or stream cross-section on graph paper as shown in Figure 4.10.



- To get the cross-sectional area of the stream flow simply. Measure water depth at two foot intervals,
- Mark your measuring stick in 3 inch intervals or every 2.5 feet from top to bottom.
- Each "square" of water depth you measure will have an area 0.5 square feet!
- All you have to do is count your squares and multiply by 0.5 to get your area in square feet.
- Count squares more than half as one.

TDSTestr™ with ATC

Full readout pocket size TDS and conductivity testers

BEFORE FIRST USE: Remove plastic strips between batteries and contacts if present (see box side panels). Soak electrodes for a few minutes in alcohol to remove oils.

CAUTION: TO AVOID CROSS CONTAMINATION BETWEEN SAMPLES, NEVER IMMERSE THE TDSTESTR ABOVE THE COLOR BAND!

CALIBRATION: Using the guide on the side box panel, select a calibration standard appropriate for your TDSTestr model. When selecting a TDS standard for ppt or ppm TDS readings it is best to select a standard having a similar chemical make-up as the test solution. Best accuracy is obtained when the calibration solution value is close to the test solution value.

Pour the calibration standard into two separate containers and some tap or deionized water into a third container. Rinse the electrode in the water, rinse it in first container of standard, then dip it into the second, turn on the TDSTestr and allow the display to stabilize. Using a small screwdriver, adjust the trimmer located on the back of the unit until the display reads the same value as the standard. Rinse the electrode in the tap or deionized water and proceed with testing.

TDS or CONDUCTIVITY TESTING:

- Remove cap.
- Press ON/OFF button to turn on.
- Dip the electrode end into the test solution.
- Allow time for the Automatic Temperature Compensation to correct the readings for solution temperature changes.
- Note the full reading once the display stabilizes.
- Press the ON/OFF button to shut off. Replace cap.

MAINTENANCE: To improve performance, clean the stainless steel electrodes by periodically rinsing them in alcohol for 10-15 minutes. Replace all 4 batteries if the display becomes faint or disappears, or it the readings are unstable or never change.

CHANGING BATTERIES: Flip up the battery compartment lid (see box side panels). Remove old batteries and replace with fresh ones noting polarity as shown in the battery compartment. Note: no battery connector wires to break!

SPECIFICATIONS

MODEL #	MN-19800-00 TDSTestr 1	MN-19800-10 TDSTestr 2	MN-19800-20 TDSTestr 3	MN-19800-30 TDSTestr 4
Range	0-1990ppm	0-10.00ppt	0-1990µS	0-19.90mS
Resolution	10ppm	0.10ppt	10uS	0.10mS
Accuracy	±2%FS	±2%FS	±2%FS	±2%FS
Operating Temperature	0-50 °C, 32 to 122°F			
Temperature Compensation	Automatic (ATC), 32 to 122°F, 0 to 50°C 2% per °C, 1.11% per °F			
Battery Life	4pcs. 1.4V Eveready EP675HP/200 hours			
Dimensions	5.94"L x 1.65"W x 0.94"H			
Wetted Materials	316 Stainless Steel and glass reinforced thermoplastic polyester			
Weight	4.5 ounces/128 gm (boxed) 3.25 ounces/92 gm (unit only)			

▲ LIFT FLAP TO OPEN ▲

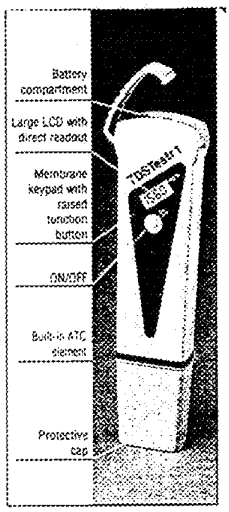
Use the guide below for selecting a calibration solution appropriate for your TDSTestr.

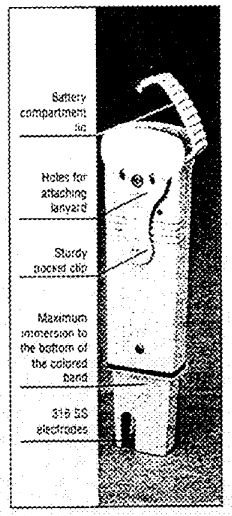
TDSTestr 1: Use a standard between 300ppm and 1990ppm.

TDSTestr 2: Use a standard between 3ppt and 10.00 ppt.

TDSTestr 3: Use a standard between 300µS and 1990µS.

TDSTestr 4: Use a standard between 3mS and 19.90mS.



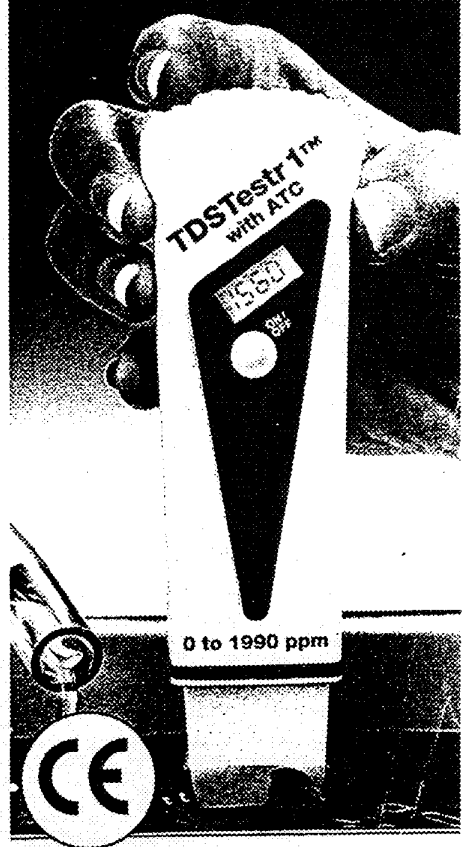

Battery compartment lid
Holes for attaching lanyard
Sturdy pocket clip
Maximum immersion to the bottom of the colored band
316 SS electrodes

Cole Parmer

COLE-PARMER INSTRUMENT COMPANY
625 East Bunker Court
Vernon Hills, IL 60061
800-323-4340

TDSTestr™

Pocket sized testers for the measurement of Total Dissolved Solids or Conductivity ...with DIRECT READOUT!



0 to 1990 ppm

- *Large LCD with direct readout— no multiplication needed!*
- *Automatic Temperature Compensation (ATC)!*
- *±2% FS accuracy!*
- *Splashproof membrane keypad*
- *Four models available featuring the most useful ranges for the widest variety of applications.*

Parameters: Methodology and Environmental Impact

The analysis of water samples from surface water (lakes, streams and rivers), groundwater and treatment facilities requires the analyst to utilize a number of tests and test methods. The sampling selected for study are intended to profile water quality in the water bodies in the project area. The Kentucky Department for Environmental Protection uses methods for analysis as approved by the United States Environmental Protection Agency (USEPA).

The parameters and test methods are shown below. Criteria and Kentucky average values for these parameters are presented in Table I.

PARAMETER	USEPA ANALYTICAL METHOD
Field Data:	
Temperature	Thermometer
pH	Colorometric
Conductivity	Specific Conductance
Dissolved Oxygen	Modified Winkler
Laboratory Analysis:	
pH	Electrometric
Total Organic Carbon	UV Promoted
Total Suspended Solids	Gravimetric
Ammonia Nitrogen	Ion Chromatography
Nitrate/Nitrite-Nitrogen	Ion Chromatography
Phosphorus, total	Spectrometric
Bromide	Ion Chromatography
Fluoride	Ion Chromatography
Sulfate	Spectrometric
Chloride	Ion Chromatography
Metal Scan (Aluminum, Barium, Cadmium, Calcium, Chromium, Iron, Lead, Magnesium, Manganese, Sodium, Zinc)	ICP
Biological Analysis	
Chlorophyll A	
Algael assays	

TABLE I

Parameters	Criteria (Limits)	KY Average Values*
Field Data		
Temperature (°C)	<22.2° C (72°F) Avg. Oct. 16-31 (WAH)	14.7°C
	<25°C (77°F) instantaneous maximum (WAH)	
pH (pH units)	6.0 - 9.0 (WAH)	7.4 units
Conductivity (Dissolved solids) (μmho/cm or mg/L)	<800 micromhos/cm (or = 500 mg/L dissolved solids) (OR)	335 μmhos/cm
Dissolved oxygen (mg/L)	>5.0 mg/L (WAH)	9.1 mg/L
	>4.0 mg/L instantaneous minimum (WAH)	
Laboratory Analysis (will include repeat of pH and conductivity)		
Total Organic Carbon (mg/L)	NC	3.5 mg/L
Total Suspended Solids (mg/L)	NC	42.4 mg/L
Bromides (mg/L)	NC	42.4 mg/L
Ammonia Nitrogen (mg/L)	<0.05 mg/L unionized NH_3 (WAH) <6.4 mg/L Total NH_3/NH_4^+N (at pH 7.4 and 15°C)	0.15 mg/L
Nitrate/Nitrite-Nitrogen (mg/L)	<10 mg/L (DWS)	0.85 mg/L
Phosphorus, total (mg/L)	NC	0.15 mg/L
Sulfate (mg/L)	<250 mg/L (DWS)	63.3 mg/L

Parameters	Criteria (Limits)	KY Average Values*
Chloride (mg/L)	<250 mg/L (DWS)	13.2 mg/L
	<600 mg/L (WAH)	
Fluoride (mg/L)	<1.0 mg/L (DWS)	Not available
Metal Scan		
Calcium (mg/L)	NC	39.9 mg/L
Magnesium (mg/L)	NC	10.6 mg/L
Iron (mg/L)	<1.0 mg/L (WAH)	1.34 mg/L
Aluminum (mg/L)	NC	0.7 mg/L
Barium (mg/L)	<1.0 mg/L (DWS)	0.05 mg/L
Cadmium (mg/L)	<0.001 mg/L (WAH) (calculated at hardness 100) <0.01 mg/L (DWS)	0.0014 mg/L
Chromium (mg/L)	<0.011 mg/L (WAH) (calculated at hardness 100) <0.05 mg/L (DWS)	0.0026 mg/L
Lead (mg/L)	0.003 mg/L (WAH) (calculated at hardness 100) <0.05 mg/L (DWS)	0.0127 mg/L
Manganese (mg/L)	<0.05 mg/L (DWS)	0.211 mg/L
Sodium (mg/L)	NC	14.8 mg/L
Biological		
Chlorophyll A	NC	
Algal Assays	NC	

CRITERIA:

WAH = Warm Water Aquatic Habitat
PCR = Primary Contact Recreation (swimming)
NC = No Criteria
DWS = Domestic Water Supply
OR = Ohio River
SCR = Secondary Contact Recreation (boating and fishing)

* Source: Division of Water Ambient Monitoring Database, 1985 - 1994.

Temperature

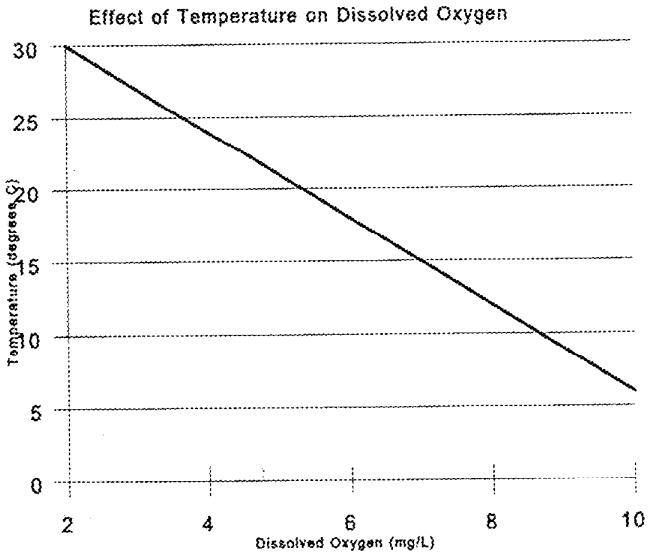
In addition to having its own toxic effect, temperature affects the solubility and, in turn, the toxicity of many other parameters. Generally the solubility of solids increases with increasing temperature, while gases tend to be more soluble in cold water. Temperature is a factor in determining allowable limits for other parameters such as ammonia.

Methodology: The simplest field method is to use a thermometer; however, electronic thermal sensing devices are available with continuous read-outs.

Environmental Impact: The Federal Water Pollution Control Administration (1967) referred to temperature as "a catalyst, a depressant, an activator, a restrictor, a stimulator, a controller, a killer, one of the most important and most influential water quality characteristics to life in water."

An important physical relationship exists between the amount of dissolved oxygen in a body of water and its temperature. Simply put, "the warmer the water, the *less* dissolved oxygen, and vice versa." Figure 2 shows the relationship.

Figure 2



For this reason, heat or "thermal pollution" may be a problem, especially in shallow slow-moving streams, embayments, or pools which can get very warm in mid-summer.

SAMPLE COLLECTION METHODS FOR IMMUNOASSAY TESTING

CHECKLIST:

_____Chain of Custody Record (Field Data Sheet)

_____Pre-cleaned amber glass bottle with Teflon-lined screw cap

_____Water-proof pen (Scripie)

_____Ice (Cooler or bucket)

_____Waders and gloves as desired

COLLECTION METHOD

Avoid disturbing sediment layer. If you are wading into the stream, allow the water to clear before you take the sample.

Face upstream (water is running toward you) to sample so that contaminants from your hands will not be collected.

Collect the sample only in the pre-cleaned amber glass bottle provided. DO NOT use any plastic container. Rinse the collection bottle three times in the water to be sampled before the final fill. Fill with mouth of bottle facing upstream away from your body or hands.

Fill the bottle (no air space) if at all possible; Cap with the Teflon-lined screw cap provided.

Complete information on the chain of custody record. Be sure the sample number on the chain of custody Record matches the sample number on the lid of the bottle.

Chill on ice immediately; keep refrigerated or on ice until the sample reaches the lab.

CHAIN OF CUSTODY:

- Complete chain of custody record for each sample taken.
- Be sure your sample code is indicated in the upper right hand corner of the Chain of Custody Record.
- Please include a complete site description with reference points you would find on a topo map
- Sign the "Relinquished by" portion and witness the individual receiving the sample signing the "Received by" section.
- Take your sample directly to the lab at the UK Mines and Minerals Building, Rose Street 3rd Floor. Call for an appt before you go, or make arrangements with your collection coordinator listed below.

Important Phone Numbers:

1-800-928-0045 Ext. 473	**Volunteer Support**	**Lab: 606-257-5500**
(606) 846-4905	**Project Headquarters**	**Dr Francis or Alice Shelly**

Watershed Watch Chain of Custody Record

Sample #	Stream Name	Date:	Time:
		Supervising Sampler ☐ Check if Correct	
Watershed	Sampling Location		
		Telephone:	

Description of General Water Conditions

Check Flow Rate	Rain in last 24 hrs?	Water Chemistry			Containers	
		Oxygen	pH	Temp		
☐ 0-Dry	☐ zero				I	
☐ 1-Ponded	☐ <.1"				N	
☐ 2-Low	☐ <.5"				S	
☐ 3-Normal	☐ <1.0"					
☐ 4-Bank Full	☐ <1.5"					
☐ 5-Flood!	☐ Gullywasher!					

General comments, questions, concerns or suggestions.

When transporting samples to the lab, it is necessary to have each person that controls the sample to sign when they receive it AND when they relinquish it.

Relinquished by:	Time/Date	Received by:	Time/Date

This form must accompany your sample. Make a copy for yourself, then send the original on its way with your sample runner. If you have questions or difficulties, please contact us at 1-800-928-0045 Ext 473

SURFACE-WATER STATIONS, IN DOWNSTREAM ORDER, FOR WHICH RECORDS ARE PUBLISHED IN THIS VOLUME

[Letters after station name designate type of data: (d) discharge, (g) stage, (c) chemical, (b) biological, (t) water temperature, (s) sediment]

	STATION NUMBER	Page
OHIO RIVER BASIN		
Ohio River:		
BIG SANDY RIVER BASIN		
Levisa Fork (head of Big Sandy River):		
Grapevine Creek near Phyllis (d)	03207965	39
Russell Fork at Haysi, VA (d)	03208500	40
Levisa Fork at Pikeville (d)	03209500	41
Johns Creek near Meta (d)	03210000	42
Levisa Fork at Paintsville (d)	03212500	43
LITTLE SANDY RIVER BASIN		
Little Sandy River at Grayson (d)	03216500	44
Ohio River at Greenup Dam (d)	03216600	45
TYGARTS CREEK BASIN		
Tygarts Creek near Greenup (d)	03217000	53
KINNICONICK CREEK BASIN		
Kinniconick Creek at Tannery (d)	03237250	54
LICKING RIVER BASIN		
Licking River near Salyersville (d)	03248500	55
Fox Creek:		
Rock Lick Creek above Unnamed Tributary near Sharkey (d)	03250310	56
Rock Lick Creek at State Highway 158 near Sharkey (d)	03250322	57
North Fork Licking River near Mt. Olivet (d)	03251200	58
South Fork Licking River:		
Hinkston Creek near Carlisle (d)	03252300	59
Licking River at Catawba (d)	03253500	60
Ohio River at Markland Dam (d)	03277200	61
KENTUCKY RIVER BASIN		
North Fork Kentucky River (head of Kentucky River):		
Leatherwood Creek at Daisy (d)	03277400	62
North Fork Kentucky River at Jackson (d)	03280000	63
Cutshin Creek at Wooton (d)	03280700	64
Middle Fork Kentucky River at Tallega (d)	03281000	65
Kentucky River:		
Red Bird River (head of South Fork Kentucky River) near Big Creek (d)	03281040	66
Goose Creek at Manchester (d)	03281100	67
South Fork Kentucky River at Booneville (d)	03281500	68
Kentucky River at lock 14, at Heidelberg (d)	03282000	69
Sturgeon Creek at Cressmont (d)	03282040	70
Red River near Hazel Green (d)	03282500	71
Red River at Clay City (d)	03283500	72
Kentucky River at lock 10, near Winchester (d)	03284000	73
Dix River near Danville (d)	03285000	74
Clarks Run near Danvile (d)	03285200	75
Kentucky River at lock 7, near High Bridge (d)	03286500	76
Kentucky River at lock 6, near Salvisa (d)	03287000	77
Kentucky River at lock 4, at Frankfort (d)	03287500	78
Elkhorn Creek:		
North Elkhorn Creek near Georgetown (d)	03288000	79
North Elkhorn Creek at Georgetown (d)	03288100	80
Royal Spring at Georgetown (d)	03288110	81
South Elkhorn Creek near Midway (d)	03289300	82
Elkhorn Creek near Frankfort (d)	03289500	83
Kentucky River at lock 2, at Lockport (d)	03290500	84
Eagle Creek at Glencoe (d)	03291500	85
HARRODS CREEK BASIN		
Harrods Creek near Prospect (c)	03292473	86

SURFACE-WATER STATIONS, IN DOWNSTREAM ORDER, FOR WHICH RECORDS ARE PUBLISHED IN THIS VOLUME--Continued

[Letters after station name designate type of data: (d) discharge, (g) stage, (c) chemical, (b) biological, (t) water temperature, (s) sediment]

	STATION NUMBER	Page
OHIO RIVER BASIN--Continued		
Ohio River--Continued		
GOOSE CREEK BASIN		
Goose Creek at Old Westport Road near St. Matthews (d, c)	03292474	87
Goose Creek at U.S. Hwy 42 near Glenview Acres (c)	03292475	89
Little Goose Creek near Harrods Creek (c)	03292480	90
BEARGRASS CREEK BASIN		
South Fork Beargrass Creek at Louisville (d,c)	03292500	91
South Fork Beargrass Creek at Winter Avenue at Louisville (c)	03292550	93
Middle Fork Beargrass Creek at Louisville (d,c)	03293000	94
Middle Fork Beargrass Creek at Scenic Loop at Louisville (c)	03293200	96
Muddy Fork at Mockingbird Valley Road at Louisville (c)	03293530	97
Ohio River at Louisville (d)	03294500	98
MILL CREEK BASIN		
Mill Creek Cutoff near Louisville (c)	03294550	99
Mill Creek at Orell Road near Louisville (c)	03294570	100
SALT RIVER BASIN		
Salt River at Glensboro (d)	03295400	101
Brashears Creek at Taylorsville (d)	03295890	102
Floyds Fork:		
Floyds Fork near Pewee Valley (d)	03297900	103
Long Run near Fisherville (c)	03297980	104
Floyds Fork at Fisherville (d,c)	03298000	105
Pope Lick at Pope Lick Road near Middletown (c)	03298100	107
Chenoweth Run at Gelhaus Lane near Fern Creek (c)	03298150	108
Floyds Fork near Mount Washington (c)	03298200	109
Cedar Creek at Fairmount Road near Mt. Washington, Ky. (d, c)	03298242	110
Cedar Creek at Thixton Road near Louisville (c)	03298250	116
Pennsylvania Run at Mt. Washington Road near Louisville (c)	03298300	117
Salt River at Shepherdsville (d)	03298500	118
Long Lick near Clermont (d)	03298550	119
Rolling Fork:		
Beech Fork at Maud (d)	03300400	120
Rolling Fork near Boston (d)	03301500	121
Wilson Creek at Harrison Fork Road near Deatsville (c)	03301575	122
Pond Creek:		
Southern Ditch:		
Southern Ditch at Minors Lane near Okolona (c)	03301880	123
Slop Ditch near Okolona (d)	03301885	124
Northern Ditch:		
Fern Creek at Old Bardstown Road at Louisville (c)	03301900	127
Northern Ditch at Okolona (c)	03301940	128
Spring Ditch at Private Drive near Okolona (c)	03301950	129
Pond Creek near Louisville (d,c)	03302000	130
Pond Creek at Pendleton Road near Louisville (c)	03302030	132
OTTER CREEK BASIN		
Otter Creek at Otter Creek Park near Rock Haven, Ky. (c)	03302110	133
Ohio River at Cannelton Dam (d,c)	03303280	134
GREEN RIVER BASIN		
Green River:		
Russell Creek near Columbia (d)	03307000	142
Green River at Munfordville (d)	03308500	143
Nolin River at White Mills (d)	03310300	144
Nolin River at Kyrock (d)	03311000	145
Beaver Creek at Hwy 31 E near Glasgow (d)	03312765	146
Barren River:		
West Fork Drakes Creek near Franklin (d)	03313700	147
Green River at Paradise (d)	03316500	148

SURFACE-WATER STATIONS, IN DOWNSTREAM ORDER, FOR WHICH RECORDS ARE PUBLISHED IN THIS VOLUME--Continued

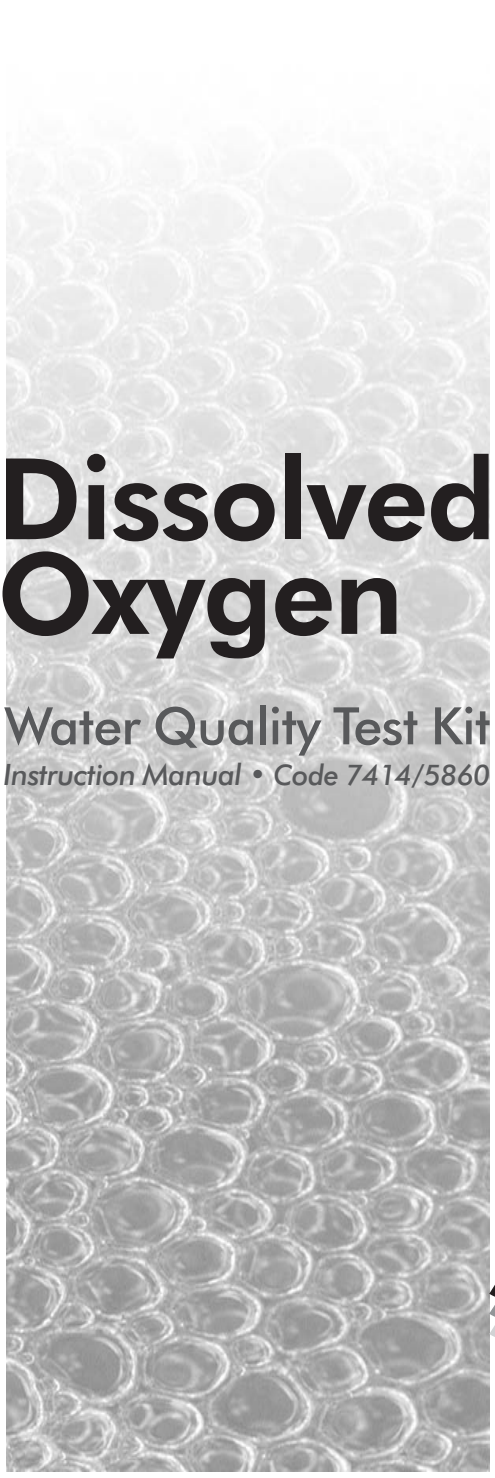
[Letters after station name designate type of data: (d) discharge, (g) stage, (c) chemical, (b) biological, (t) water temperature, (s) sediment]

	STATION NUMBER	Page
OHIO RIVER BASIN--Continued		
Ohio River--Continued		
GREEN RIVER BASIN--Continued		
Green River at lock 2, at Calhoun (d)	03320000	149
Pond River near Apex (d)	03320500	150
WABASH RIVER BASIN		
Wabash River at New Harmony, IN (c)	03378500	152
TRADEWATER RIVER BASIN		
Tradewater River at Olney (d)	03383000	159
CUMBERLAND RIVER BASIN		
Martins Fork Lake at Martins Fork Dam near Smith (c,t)	03400798	160
Martins Fork near Smith (d,c,t)	03400800	183
Cumberland River near Harlan (d)	03401000	190
Yellow Creek near Middlesboro (d)	03402000	191
Cumberland River at Pine St. Bridge at Pineville, KY (d)	03402900	192
Cumberland River at Barbourville (d)	03403500	193
Clear Fork at Saxton (d)	03403910	194
Cumberland River at Williamsburg (d)	03404000	195
Laurel River:		
Lynn Camp Creek at Corbin (d)	03404900	196
Rockcastle River at Billows (d)	03406500	197
South Fork Cumberland River near Stearns (d)	03410500	198
Beaver Creek near Monticello (d)	03413200	199
Little River near Cadiz (d)	03438000	200
Barkley-Kentucky Canal near Grand Rivers (d)	03438190	201
Cumberland River near Grand Rivers (d)	03438220	202
TENNESSEE RIVER BASIN		
Tennessee River at Hwy 60, near Paducah, Ky (c)	03609750	203
Clarks River at Almo (d)	03610200	210
MASSAC CREEK BASIN		
Massac Creek near Paducah (d)	03611260	211
Ohio River at Metropolis, IL (d)	03611500	212
BAYOU CREEK BASIN		
Bayou Creek:		
Bayou Creek near Heath (d)	03611800	213
Bayou Creek near Grahamville (d)	03611850	214
Little Bayou Creek near Grahamville (d)	03611900	215
Ohio River at lock and dam 53, near Grand Chain, IL (c)	03612500	216
LOWER MISSISSIPPI RIVER BASIN		
BAYOU DE CHIEN BASIN		
Bayou De Chien near Clinton (d)	07024000	223

Dissolved Oxygen

Water Quality Test Kit

Instruction Manual • Code 7414/5860



LaMotte

INTRODUCTION

Aquatic animals need dissolved oxygen to live. Fish, invertebrates, plants, and aerobic bacteria all require oxygen for respiration. Oxygen dissolves readily into water from the atmosphere until the water is saturated. Once dissolved in the water, the oxygen diffuses very slowly and distribution depends on the movement of the aerated water. Oxygen is also produced by aquatic plants, algae, and phytoplankton as a by-product of photosynthesis.

The amount of oxygen required varies according to species and stage of life. Dissolved Oxygen levels below 3 ppm are stressful to most aquatic organisms. Dissolved Oxygen levels below 2 or 1 ppm will not support fish. Levels of 5 to 6 ppm are usually required for growth and activity.

This test kit uses the azide modification of the Winkler method for determining dissolved oxygen.

TABLE OF CONTENTS

Page

Kit Contents 2

Test Procedure

Part 1: Collecting a Water Sample 3

Part 2: Adding the Reagents 4

Part 3: Titration 5

EPA Compliance 8

Dissolved Oxygen Fact Sheet 10

General Safety Precautions 13

Use Proper Analytical Techniques 14

Material Safety Data Sheets 15

Kit Diagrams 21

Short Form Instructions ..... **Back Cover**

WARNING! This set contains chemicals that may be harmful if misused. Read cautions on individual containers carefully. Not to be used by children except under adult supervision

KIT CONTENTS

QUANTITY	CONTENTS	CODE
30 mL	*Manganous Sulfate Solution	*4167-G
30 mL	*Alkaline Potassium Iodide Azide	*7166-G
50 g	*Sulfamic Acid Powder (7414 Kit)	*6286-H
30 mL	*Sulfuric Acid, 1:1 (5860 Kit)	*6141WT-G
60 mL	*Sodium Thiosulfate, 0.025N	*4169-H
30 mL	Starch Indicator Solution	4170WT-G
1	Spoon, 1.0 g, plastic (7414 Kit)	0697
1	Direct Reading Titrator	0377
1	Test Tube, 5-10-12.9-15-20-25 mL, glass, w/cap	0608
1	Water Sampling Bottle, 60 mL, glass	0688-DO

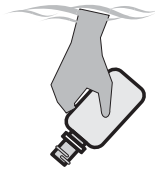
***WARNING:** Reagents marked with a * are considered to be potential health hazards. To view or print a Material Safety Data Sheet (MSDS) for these reagents see MSDS CD or www.lamotte.com. To obtain a printed copy, contact LaMotte by email, phone or fax.

To order individual reagents or test kit components, use the specified code numbers.

TEST PROCEDURE

PART 1 - COLLECTING THE WATER SAMPLE

1.



Rinse the Water Sampling Bottle (0688-DO) with the sample water.

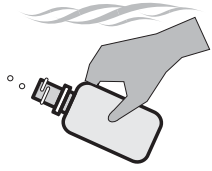
2.

Tightly cap the bottle, and submerge it to the desired depth.

3.

Remove the cap and allow the bottle to fill.

4.

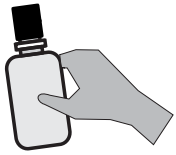


Tap the sides of the bottle to dislodge any air bubbles.

5.

Replace the cap while the bottle is still submerged.

6.



Retrieve the bottle and make sure that no air bubbles are trapped inside.

TEST PROCEDURE

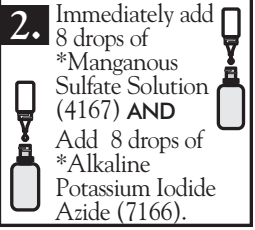
PART 2 - ADDING THE REAGENTS

NOTE:

Be careful not to introduce air into the sample while adding the reagents.

1. Remove the cap from the bottle.

2. Immediately add 8 drops of *Manganous Sulfate Solution (4167) **AND** Add 8 drops of *Alkaline Potassium Iodide Azide (7166).



3.

Cap the bottle and mix by inverting several times. A precipitate will form.

4.

Allow the precipitate to settle below the shoulder of the bottle.

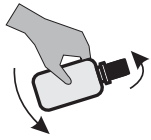
5.

For Kit Code 7414:
Immediately use the 1.0 g spoon (0697) to add one level measure of *Sulfamic Acid Powder (6286).

OR

For Kit Code 5860:
Add 8 drops of *Sulfuric Acid, 1:1 (6141WT).

6. Cap and gently invert the bottle to mix the contents until the precipitate and the reagent have totally dissolved. The solution will be clear yellow to orange if the sample contains dissolved oxygen.



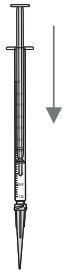
NOTE: At this point the sample has been "fixed" and contact between the sample and the atmosphere will not affect the test result. Samples may be held at this point and titrated later.

TEST PROCEDURE

PART 3 - THE TITRATION

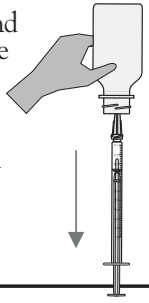
1. Fill the titration tube (0608) to the 20 mL line with the fixed sample. Cap the tube.

2. Depress plunger of the Titrator (0377).



3. Insert the Titrator into the plug in the top of the *Sodium Thiosulfate, 0.025N (4169) titrating solution.

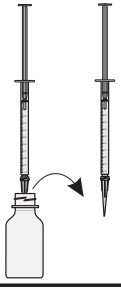
4. Invert the bottle and slowly withdraw the plunger until the large ring on the plunger is opposite the zero (0) line on the scale.



NOTE:

If small air bubbles appear in the Titrator barrel, expel them by partially filling the barrel and pumping the titration solution back into the reagent container. Repeat until bubble disappears.

5. Turn the bottle upright and remove the Titrator.



NOTE:

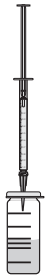
If the sample is a very pale yellow, go to Step 9.

continued . . .

TEST PROCEDURE

6.

Insert the tip of the Titrator into the opening of the titration tube cap.



7.

Slowly depress the plunger to dispense the titrating solution until the yellow-brown color changes to a very pale yellow. Gently swirl the tube during the tiration to mix the contents.

8.

Carefully remove the Titrator and cap. Do not to disturb the Titrator plunger.

9.

Add 8 drops of Starch Indicator Solution (4170WT). The sample should turn blue.

10.

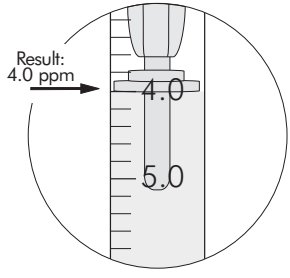
Cap the titration tube. Insert the tip of the Titrator into the opening of the titration tube cap.

11.

Continue titrating until the blue color disappears and the solution becomes colorless.

12.

Read the test result directly from the scale where the large ring on the Titrator meets the Titrator barrel. Record as ppm Dissolved Oxygen. Each minor division on the Titrator scale equals 0.2 ppm.



TEST PROCEDURE

NOTE:

If the plunger ring reaches the bottom line on the scale (10 ppm) before the endpoint color change occurs, refill the Titrator and continue the titration. Include the value of the original amount of reagent dispensed (10 ppm) when recording the test result.

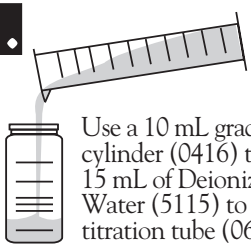
NOTE:

When testing is complete, discard titrating solution in Titrator. Rinse Titrator and titration tube thoroughly. DO NOT remove plunger or adapter tip.

EPA COMPLIANCE

To qualify as an EPA accepted test, and to achieve the greatest accuracy, the Sodium Thiosulfate Solution, 0.025N (4169) must be standardized daily. This procedure follows Standard Methods for the Examination of Water and Wastewater. Numbers in ( ) are for LaMotte products. These products are not included in this kit but can be ordered from LaMotte Company by using the specified code number.

1.



Use a 10 mL graduated cylinder (0416) to add 15 mL of Deionized Water (5115) to the titration tube (0608).

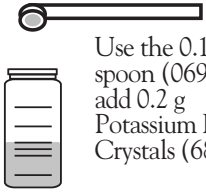
2.

Use a Direct Reading Titrator, 0-1 Range (1.0 mL capacity) (0376) to add 2 mL of Potassium Bi-iodate (7346).

3.

Add 2 drops of Sulfuric Acid, 5N (8517WT).

4.

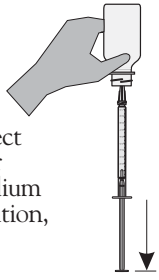


Use the 0.1 g spoon (0699) to add 0.2 g Potassium Iodide Crystals (6809).

5.

Swirl to dissolve. Solution will turn yellowish brown.

6.



Fill another Direct Reading Titrator (0376) with Sodium Thiosulfate Solution, 0.025N (4169).

EPA COMPLIANCE

7.

While gently swirling the tube, add Sodium Thiosulfate, 0.025N until the color fades to pale yellow. It will be necessary to refill the Direct Reading Titrator.

8.

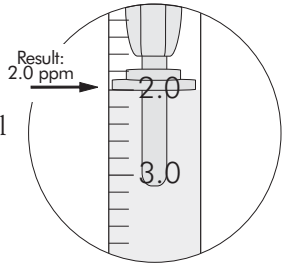
Add 3 drops of Starch Indicator Solution (4170WT). The solution will turn blue.

9.

Continue adding Sodium Thiosulfate, 0.025N until the blue color disappears and the solution is colorless.

10.

Read the test result directly from the scale where the large ring on the Titrator meets the Titrator barrel. Include the value of the original amount dispensed (1 mL). If the reading is 2.0 +/-0.1 mL, the Sodium Thiosulfate, 0.025N (4169) is satisfactory. If not, discard and replace with new reagent.



DISSOLVED OXYGEN FACT SHEET

Oxygen is critical to the survival of aquatic plants and animals, and a shortage of dissolved oxygen is not only a sign of pollution, it is harmful to fish. Some aquatic species are more sensitive to oxygen depletion than others, but some general guidelines to consider when analyzing test results are:

5–6 ppm	**Sufficient for most species**
<3 ppm	**Stressful to most aquatic species**
<2 ppm	**Fatal to most species**

Because of its importance to the fish's survival, aquaculturists, or "fish farmers," and aquarists use the dissolved oxygen test as a primary indicator of their system's ability to support healthy fish.

WHERE DOES THE OXYGEN COME FROM?

The oxygen found in water comes from many sources, but the largest source is oxygen absorbed from the atmosphere. Wave action and splashing allows more oxygen to be absorbed into the water. A second major source of oxygen is aquatic plants, including algae; during photosynthesis plants remove carbon dioxide from the water and replace it with oxygen.

Absorption

Oxygen is continuously moving between the water and surrounding air. The direction and speed of this movement is dependent upon the amount of contact between the air and water. A tumbling mountain stream or windswept, wave-covered lake, where more of the water's surface is exposed to the air, will absorb more oxygen from the atmosphere than a calm, smooth body of water. This is the idea behind aerators: by creating bubbles and waves the surface area is increased and more oxygen can enter the water.

Photosynthesis

In the leaves of plants, one of the most important chemical processes on Earth is constantly occurring: photosynthesis. During daylight, plants constantly take carbon dioxide from the air, and in the presence of water convert it to oxygen and carbohydrates, which are used to produce additional plant material. Since photosynthesis requires light, plants do not photosynthesize at night, so no oxygen is produced. Chemically, the photosynthesis reaction can be written as:

$$\text{Light} + n\text{CO}_2 + n\text{H}_2\text{O} \longrightarrow (\text{C}_2\text{HO})n + n\text{O}_2$$

Light + Carbon Dioxide + Water ⟶ Carbohydrate + Oxygen

WHERE DOES THE OXYGEN GO?

Once in the water, oxygen is used by the aquatic life. Fish and other aquatic animals need oxygen to breathe or respire. Oxygen is also consumed by bacteria to decay, or decompose, dead plants and animals.

Respiration

All animals, whether on land or underwater, need oxygen to respire, grow and survive. Plants and animals respire throughout the night and day, consuming oxygen and producing carbon dioxide, which is then used by plants during photosynthesis.

Decomposition

All plant and animal waste eventually decomposes, whether it is from living animals or dead plants and animals. In the decomposition process, bacteria use oxygen to oxidize, or chemically alter, the material to break it down to its component parts. Some aquatic systems may undergo extreme amounts of oxidation, leaving no oxygen for the living organisms, which eventually leave or suffocate.

OTHER FACTORS

The oxygen level of a water system is not only dependent on production and consumption. Many other factors work together to determine the potential oxygen level, including:

- **Salt vs. fresh water** - Fresh water can hold more oxygen than salt water.
- **Temperature** - Cold water can hold more oxygen than warm water.
- **Atmospheric pressure (Altitude)** - The greater the atmospheric pressure the more oxygen the water will hold.

TESTING DISSOLVED OXYGEN

Dissolved oxygen is often tested using the Azide modification of the Winkler method. When testing dissolved oxygen it is critical not to introduce additional oxygen into the sample. Many people avoid this problem by filling the sample bottle all the way and allowing the water to overflow for one minute before capping.

The first step in a DO titration is the addition of Manganous Sulfate Solution (4167) and Alkaline Potassium Iodide Azide Solution (7166). These reagents react to form a white precipitate, or floc, of manganous hydroxide, $Mn(OH)_2$. Chemically, this reaction can be written as:

$$MnSO_4 + 2KOH \longrightarrow Mn(OH)_2 + K2SO_4$$

Manganous Sulfate + Potassium Hydroxide ⟶ Manganous Hydroxide + Potassium Sulfate

Immediately upon formation of the precipitate, the oxygen in the water oxidizes an equivalent amount of the manganous hydroxide to brown-colored manganic hydroxide. For every molecule of oxygen in the water, four molecules of manganous hydroxide are converted to manganic hydroxide. Chemically, this reaction can be written as:

$$4Mn(OH)_2 + O_2 + 2H_2O \longrightarrow 4Mn(OH)_3$$

Manganous Hydroxide + Oxygen + Water ⟶ Manganic Hydroxide

After the brown precipitate is formed, a strong acid, such as Sulfamic Acid Powder (6286) or Sulfuric Acid, 1:1 (6141) is added to the sample. The acid converts the manganic hydroxide to manganic sulfate. At this point the sample is considered "fixed" and concern for additional oxygen being introduced into the sample is reduced. Chemically, this reaction can be written as:

$$2Mn(OH)_3 + 3H_2SO_4 \longrightarrow Mn_2(SO_4)_3 + 6H_2O$$

Manganic Hydroxide + Sulfuric Acid ⟶ Manganic Sulfate + Water

Simultaneously, iodine from the potassium iodide in the Alkaline Potassium Iodide Azide Solution is oxidized by manganic sulfate, releasing free iodine into the water. Since the manganic sulfate for this reaction comes from the reaction between the manganous hydroxide and oxygen, the amount of iodine released is directly proportional to the amount of oxygen present in the original sample. The release of free iodine is indicated by the sample turning a yellow-brown color. Chemically, this reaction can be written as:

$$Mn_2(SO_4)_3 + 2KI \longrightarrow 2MnSO_4 + K_2SO_4 + I_2$$

Manganic Sulfate + Potassium Iodide ⟶ Manganous Sulfate + Potassium Sulfate + Iodine

The final stage in the Winkler titration is the addition of sodium thiosulfate. The sodium thiosulfate reacts with the free iodine to produce sodium iodide. When all of the iodine has been converted the sample changes from yellow-brown to colorless. Often a starch indicator is added to enhance the final endpoint. Chemically, this reaction can be written as:

$$2Na_2S_2O_3 + I_2 \longrightarrow Na_2S_4O_6 + 2NaI$$

Sodium Thiosulfate + Iodine ⟶ Sodium Tetrathionate + Sodium Iodide

GENERAL SAFETY PRECAUTIONS

1.

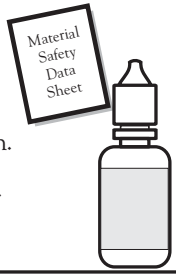
Store the test kit in a cool, dry area.

2.

Read all instructions and note precautions before performing the test procedure.

3.

Read the labels on all reagent bottles. Note warnings and first aid information. Read all Material Safety Data Sheets.



4.

Keep all equipment and reagent chemicals out of the reach of young children.

5. Avoid contact between reagent chemicals and skin, eyes, nose, and mouth.

6. Wear safety glasses when performing test procedures.

7.

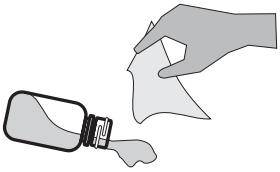
In the event of an accident or suspected poisoning, immediately call the Poison Center phone number in the front of your local telephone directory or call a physician. Additional information for all LaMotte reagents is available in the United States, Canada, Puerto Rico, and the US Virgin Islands from Chem-Tel by calling 1-800-255-3924. For other areas, call 813-248-0585 collect to contact Chem-Tel's International access number. Each reagent can be identified by the four digit number listed on the upper left corner of the reagent label, in the contents list and in the test procedures.

USE PROPER ANALYTICAL TECHNIQUES

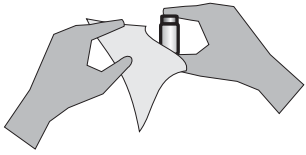
1. Use test tube caps or stoppers, not your fingers, to cover tubes during shaking or mixing.

2. Hold dropper bottles vertically upside-down, and not at an angle, when dispensing a reagent. Squeeze the bottle gently to dispense the reagent one drop at a time.

3. Wipe up any reagent chemical spills immediately.



4. Thoroughly rinse test tubes before and after each test.

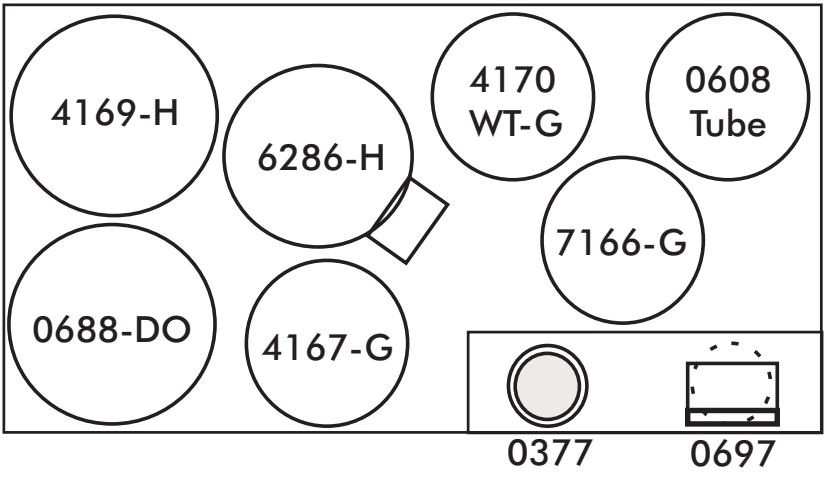


5. Tightly close all containers immediately after use. Do not interchange caps from containers.

6. Avoid prolonged exposure of equipment and reagents to direct sunlight. Protect reagents from extremes of temperature.

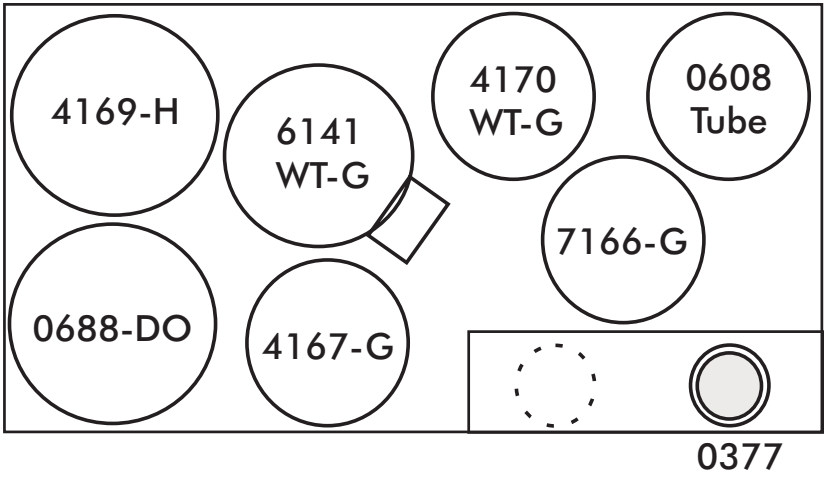
DISSOLVED OXYGEN KIT · CODE 7414

Instructions



DISSOLVED OXYGEN KIT · CODE 5860

Instructions



SHORT FORM INSTRUCTIONS

Read all instructions before performing test. Use this guide as a quick reference.

1. Fill Water Sampling Bottle (0688-DO).
2. Add 8 drops of *Manganous Sulfate Solution (4167).
3. Add 8 drops of *Alkaline Potassium Iodide Azide (7166).
4. Cap and mix.
5. Allow precipitate to settle.
6. Use the 1.0 g spoon to add *Sulfamic Acid Powder (6286) or add 8 drops of Sulfuric Acid, 1:1 (6141WT).
7. Cap and mix until reagent and precipitate dissolve.
8. Fill test tube (0608) to the 20 mL line.
9. Fill Titrator with *Sodium Thiosulfate, 0.025N (4169).
10. Titrate until sample color is pale yellow. DO NOT DISTURB TITRATOR.
11. Add 8 drops of Starch Indicator (4170WT).
12. Continue titration until blue color just disappears and solution is colorless.
13. Read result in ppm Dissolved Oxygen.

LaMOTTE COMPANY
Helping People Solve Analytical Challenges®
PO Box 329 • Chestertown • Maryland • 21620 • USA
800-344-3100 • 410-778-3100 (Outside U.S.A.) • Fax 410-778-6394
Visit us on the web at www.lamotte.com

67414-MN • 4/07

Watershed Watch Project Procedures: Collecting Samples for Laboratory Analysis Version 3.0 2005

Watershed Watch
Sample Collection Methods
Table of Contents

1. Introduction
   a. Background: Watershed Watch in Kentucky
   b. Overview: Watershed Watch's Synoptic Sampling Program
   c. Data Rigor
2. Training
3. Planning a Synoptic Sampling Event
   a. Sampling Event Coordinator
   b. Selection of Laboratory
4. Sampling Site Selection
   a. Objectives
   b. Maps
   c. Rationale
   d. Accessibility and Appropriateness
   e. Health and Safety
5. Location and Description of Sampling Sites
   a. Diagram Depicting Physical Setting
   b. Coordinates
   c. Photographs
6. Sample Materials
   a. Containers and Preservatives
   b. Preprinted Forms and Instructions
7. Sampling Procedures
   a. Completing the "Chain of Custody" form
   b. In-stream sampling Location and Approach
   c. Sampling for Parameters other than Bacteria
   d. Sampling for bacteria
   e. Sample Preservation
   f. Chemical Treatment
8. Transport of Samples to the Laboratory
   a. Documenting changes in custody of the sample
   b. Drop-Off Centers
   c. Runners
9. Laboratory Analysis
10. Data Management
11. Quality Control and Assurance
   a. How Watershed Watch Assures the Quality of Its Data
   b. Duplicate Samples and Field Blanks

1. Introduction

This document describes standard operating procedures of Watershed Watch in Kentucky for deriving reliable data about water quality samples obtained from streams for laboratory analysis by its Volunteer Monitor participants. This section of the document will provide background on the program, explain its organization, and introduce its sampling programs.

a. Background: Watershed Watch in Kentucky

Watershed Watch in Kentucky educates citizens in watershed and stream science and trains them to gather scientific data about streams.

Watershed Watch has these statewide program objectives:

- Provide citizens with an array of scientific data and an understanding of stream science that helps them better appreciate the quality status of a stream for which they are concerned;
- Create an annual synopsis of the overall quality of surface waters on a state and basin basis; and
- Involve citizens who are knowledgeable about water quality in watershed planning, protection, and restoration activities.

b. Overview: Watershed Watch's Synoptic Sampling Program

A major purpose of the Synoptic Assessment program of Watershed Watch is to generate data that volunteer monitors may use to assess conditions of the stream that most interests them.

Watershed Watch trains volunteer monitors to collect the following data streamside:

- Water Quality characteristics measured at the stream including dissolved oxygen, pH, and temperature and, in places, specific conductance;
- Physical characterization of representative stream reaches;
- Biological sampling; and
- Habitat assessment including biodiversity

This document does not discuss procedures and methods for collection of this data.

Volunteer monitors also collect grab samples about stream parameters that cannot be measured streamside and require laboratory analysis. Samples are collected for:

- Pesticides that threaten aquatic life, sampled in the Spring;
- Human pathogens (including bacteria), sampled in the Summer; and
- Major cations and anions, trace constituents, total organic carbon and other water-quality parameters, sampled in the Fall.

Methods and procedures for training volunteers to grab and transport samples, and for laboratory analysis and QA/AC, are the subject of this document.

c. Data Rigor

The KDOW has issued "Agency Guidance for Volunteer Monitoring Data and Reports" that indicates the level of procedural rigor necessary for data, depending on the intended use for the data. The guidance is summarized in the following matrix, which indicates the data-related activities that are necessary depending on the use to which volunteer-generated data will be put:

		Potential Data Rigor Requirements					
Tier	**Data Use**	Consultation with KDOW	Written Study Plan	Compliance with federal standards	SOP and QAPP pre-approved by KDOW	Use of KDOW SOP	Samplers pre-approved by KDOW
I	Incident Reporting	**X**					
	Education Programs	**X**					
	Local Awareness	**X**					
II	Watershed Screening	**X**	**X**	**O**			
	Local Planning Activities	**X**	**X**	**X**			
III	Effectiveness Monitoring	**X**	**X**	**X**	**X**	**O**	**O**
IV	TMDL Monitoring	**X**	**X**	**X**	**X**	**X**	**O**
	Use Support Determination	**X**	**X**	**X**	**X**	**X**	**X**

X = Required KDOW = Kentucky Division of Water SOP = Standard Operating Procedure
O = Optional QAPP = Quality Assurance Project Plan TMDL = Total Maximum Daily Load

Watershed Watch is designed to meet the data rigor of Tier II.

2. Training

a. Standard Sampling Curriculum

Volunteer Monitors who grab samples or supervise the sampling streamside are required to complete a Standard Sampling Training Module developed by the Training Committee and approved by the Science Advisors Committee of the ICC that addresses:

- Sample container handling
- Sample collection
- Sample preservation
- Sample transport and storage
- Documentation and chain of custody record completion
- QA/QC procedures including duplicate samples and field blanks
- Communication with Event Coordinators and lab staff.

The module includes a demonstration, ideally streamside, of sample container handling, collection, and preservation, and requires the volunteer to demonstrate competency.

3. Planning a "Synoptic" Sampling Event

a. Sampling Event Coordinator

For each synoptic sampling event, the Basin Steering Committee identifies a Sampling Event Coordinator who communicates with the receiving laboratory concerning:

- Arrangements for receiving samples (see Section 8);
- Standards for analysis (see Section 9); and
- Standard reporting spreadsheet, including flags for samples outside of standard receiving temperature and holding time ("lab report") (see Section 10)

The Sampling Event Coordinator also:

- Assembles packets of containers and corresponding instructions to Supervising Samplers;
- Makes arrangements for Drop-Off Centers and Runners as indicated;

b. Selection of laboratory

Watershed Watch uses laboratories that meet at least one of the following criteria:

- Listed on the KDOW Certified Drinking Water Lab List;
- Currently providing contract work for KDOW; or
- Approved by EPPC microbiological staff.

4. Sampling Site Selection

a. Objectives

The site selection process in Watershed Watch attempts to accommodate its two major purposes:

- The interest of the Volunteer Monitor, who often desires to focus learning activities on a stream reach where she or he lives, works, learns, or plays; and
- The need of the program and its stakeholders to collect information from a stream reach near the bottom of a watershed, where data will be most representative of the condition of the watershed.

b. Site Selection Maps

The following maps are used during training to assist Volunteer Monitors and Trainers with Site Selection:

- Kentucky Atlas and Gazetteer, ISBN Number 0-89933-216-1
- Topozone: http://www.topozone.com (Set to DD.DDDD coordinates)
- Arcview Shape Files including Counties, Roads and Streams with current Watershed Watch Site List.

c. Rationale

Because site selection in Watershed Watch attempts to balance the interest of the Volunteer Monitor in a particular stream reach with the program's objective of collecting information about watershed conditions, site selection occurs as part of Volunteer Monitor training so that trainers can encourage participants to:

- Join a team at an existing site;
- Open a new site at a location that will represent the condition of an undocumented watershed; or
- Choose a stream reach that will represent the condition of their stream of interest.

d. Accessibility and Appropriateness

The site selection unit in Watershed Watch training identifies the following factors as important in site selection:

- Proximity to existing Watershed Watch sites;
- Access to the site using public rights of way and/or the permission of the property owner;
- Physical safety in accessing the stream via the streambank;
- Wadeability of the stream;
- Representativeness of the stream reach (channel morphology and riffles)
- Reach mixing (sites near major tributaries or point sources should be avoided to minimize backwater effects or poorly mixed flows);
- Proximity to major man-made disturbances like bridges or dams; and
- Known health risks in the stream, e.g., proximity to a treatment plant or "straight pipes."

e. Health and Safety

The following health and safety factors are addressed in the training:

- Notifying others of itinerary and whereabouts;
- Never visiting an isolated site alone;
- Never sampling in high water;
- Bewaring of hunters, poisonous reptiles, and sudden high water;
- Carrying identification;
- Taking a cellular phone when available; and
- Wearing disposable, powderless gloves when handling sample preservatives such as acid.

5. Location and Description of Sampling Sites

a. Diagram depicting physical setting

The Volunteer Monitor documents the physical setting of the site using a standard form, "Physical Characterization/ Water Quality Field Data Sheet," which may be found in Appendix D.

b. Coordinates

Latitude and longitude are determined for the site in one of two ways:

- The Volunteer Monitor determines them using a handheld GPS unit and submits the coordinates with the "Physical Characterization" form. In this instance, the GPS unit must be tuned to the coordinate specifications called for in http://kywater.org/dow/gps/; or
- A copy of the 1:24,000 topographic map for the stream reach is mailed to the Volunteer Monitor with the first sampling container for the site with a request that the monitor identify the site on the map and return it to the Data Manager.

c. Photographs

The Volunteer Monitor is requested to submit two photographs of the stream reach with the "Physical Characterization" form:

- Upstream of the sample point looking downstream at the sample point (marked "downstream:); and
- Downstream of the sample point looking upstream at the sample point (marked "upstream").

6. Preparation for Sampling

a. Containers and Preservatives

Containers and preservatives for samples are obtained by Steering Committees through the ICC purchasing cooperative or independently and meet the criteria found in Appendix E, "Environmental Sample Preservation and Holding Times."

The container is pre-marked with the unique Site Number by the Sampling Event Coordinator designated by the Steering Committee before it is mailed to the Supervising Sampler for the site.

A "Chain of Custody" form (see Appendix F for the template) is prepared for each site and preprinted with the Site Number, usually by the Data Manager. It is enclosed by the Sampling Event Coordinator with a set of instructions. Samplers should open and read their packet upon receipt.

7. Sampling Procedures

The purposes of the following streamside sampling procedures are to assure that the sample container correlates with the documented sampling activity and to prevent water samples from contamination during the sampling process.

a. Completing the "Chain of Custody" form

The Watershed Watch Chain of Custody form (Appendix F) serves to document and record the transfer of the samples from the stream to the laboratory, functions as a field measurement form, and provides a place for field observations. Listed below are data elements of the form:

Sample identification: the following information is preprinted on the form by the Steering Committee's Data Manager:

- Site Number
- Stream Name
- Watershed Number
- Sampling Location
- County
- Name of and contact information for Supervising Sampler

The following identifying information is entered by the Supervising Sampler streamside when the sample is taken:

- Date and Time of sample collection
- Corrections to any preprinted information

Field measurements: The following information is entered by the Supervising Sampler when and where the sample is taken:

- Comments on general stream conditions
- Flow
- Flow Rate
- Rain in past 48 hours
- Dissolved oxygen
- pH
- Temperature
- Conductivity

Signatures: The Supervising Sampler signs the form at the time she or he relinquishes it to the laboratory or to the next person who will have custody of the sample as it is transported to the laboratory. Signatures are annotated by the date and time they are signed.

b. In-stream sampling location and approach

Samples are taken by wading to reduce sample contamination. A maximum safe wading depth depends on the size of the person sampling, the stream's velocity and depth, and the streambed material. Caution should always be used when wading streams deeper than three feet. Additional caution should be used when the streambed is composed of loose or slippery material. Algae-coated cobbles can be slippery and as dangerous as ice. A personal floatation device should be worn when wading streams three feet or greater in depth.

The sampler approaches the sampling site from a downstream location, walking upstream to the sampling site, to avoid disturbing bottom sediments that could contaminate the water quality sample. An ideal wading location is in the center of the stream and at the head of a riffle so that water current produces a good flow past the sampling point.

c. Sampling for parameters <u>other than</u> bacteria

The sampler first contaminates gloves, if worn, with stream water. Sample bottles are then contaminated with stream water. Pre-marked sample bottles are rinsed once with stream water. The sample bottle is then lowered from the surface to the bottom of the stream until the sample bottle touches the stream bottom, without disturbing sediments. Upon reaching the bottom, the bottle is raised to the surface, matching the transit rate when the bottom was lowered. Repeat until the bottle is filled with stream water. Rinse the bottle cap in the stream and cap the bottle.

d. Sampling for bacteria

The sampler first contaminates gloves, if worn, with stream water before sampling with the container. Do not pre-rinse the container, and avoid contaminating the inside of the container, especially with an ungloved finger. Dip the pre-marked sample container to a depth of about four inches with the open end facing upstream. Push the mouth of the container upstream at this depth until the container is nearly full. The mouth of the container should at all times be upstream of the sample collector and any disturbed sediments. Leave enough airspace in the top of the sample container so the sample can be remixed just before filtration at the laboratory. Immediately chill the sample in an ice slurry (see following section).

e. Sample preservation

Sample preservation procedures prevent reduction or loss of water quality variables of interest. Variable loss can occur between sample collection and laboratory analysis because of physical, chemical, and biological processes that result in chemical precipitation, absorption, oxidation, reduction, ion exchange, degassing, or degradation. Preservation stabilizes variable concentrations for a limited period of time. Some samples, particularly of bacteria, have a very short holding time before laboratory analysis may begin.

In all Watershed Watch sampling events, sample containers are placed in a container with a slurry of chilled water and ice immediately following sample collection to maintain them at 4 degrees Centigrade plus or minus 2 degrees without freezing until analyzed.

Sample preservation instructions are included with the sample bottles mailed or delivered to the Supervising Sampler prior to each sampling event.

f. Chemical treatment

If a sample requiring acidification/ chemical treatment will not be delivered to the laboratory within six hours of its collection from the stream, the following procedures are required:

Glass ampoules containing the preservative and appropriately protected for shipping are distributed by the Sampling Event Coordinator with the sampling containers and instructions to Supervising Samplers. Instructions are sent that include these precautions:

> "Preservatives in the glass ampoules are highly concentrated acids that must be handled carefully. Even a small drop of the solution can burn your skin. Use of latex gloves and safety glasses is highly recommended. Rinse each ampoule with water several times before discarding."

The following instructions are given:

1. After filling the container, carefully snap the neck of the ampoule and add it to container.
2. Label the container with the letter "N" for Nitric Acid, or "S" for Sulfuric Acid, as appropriate.
3. Place the container on ice.
4. Carefully rinse the empty preservative ampoules before discarding.

8. Transport of Samples to the Laboratory

Chilled samples should be delivered to the laboratory as soon as possible; bacteria samples, and samples that require acidification but have not been treated, must be delivered to the laboratory within six hours from the time of collection.

a. Documenting changes in custody of the sample

When the Supervising Sampler takes a sample directly to the laboratory, she or he signs, times, and dates the Chain of Custody form in the left column when custody is relinquished to the laboratory. The staff member of the laboratory who receives the sample similarly signs, times, and dates the form opposite the signature of the person relinquishing it. Identical times and dates on the same line means the sample changed custody without an intermediate step, which would disqualify the sample from regulatory use.

b. Drop-Off Centers

Because of the vast number of Watershed sites sampled on the same day and the few number of receiving laboratories, "Drop-Off Centers" may be established if these criteria are met:

- The instructions that accompany sample bottles identify the person responsible for the drop-off center, and provide directions, contact telephone number, and specific times of operation when the responsible person will be available to accept samples; and

- The center has sufficient refrigeration/cooler space to immediately chill samples transferred from Volunteer Monitors' coolers.

c. Runners

Runners may be designated to collect samples from Drop-Off Centers or Volunteer Monitors in the field. Sample runners are responsible for:

- Communicating with volunteers and drop-off locations on their route prior to sampling in order to coordinate swift collection and transfer;
- Having sufficient cooler space to immediately chill samples transferred from Volunteer Monitors' coolers;
- Communicating with the destination laboratory so it is prepared to accept the samples delivered;
- Fully understanding the delivery times required for samples; and
- Confirming that the numbering of sample containers corresponds to the number on Chain of Custody forms;
- Signing Chain of Custody Forms when receiving and relinquishing samples;
- Checking bottle caps to assure they are securely tightened (avoid over-tightening); and
- Packing samples carefully in the receiving container to prevent bottle breakage, shipping container leakage, and sample degradation.

9. Laboratory Analysis

Labs selected by Watershed Watch are asked to use standard methods of analyis.

10. Data Management

The laboratory sends its results to the project data manager. The Data Manager coordinates review of copies of the printed report by the Sampling Event Coordinator, Steering Committee Chair and Science Advisor for errors, omissions, and suitability. Draft copies are sent to supervising samplers for review and comment. Once approved by the Basin Steering Committee the monitoring data is posted on the basin web site and released at the annual conference

11. Quality Control and Assurance

a. How Watershed Watch Assures the Quality of its Data

Quality Control and Assurance (QA/QC) is the responsibility of everyone in the chain of custody of a sample, its analysis, and the data that results.

A first level of QA/QC is compliance with procedures and methods in the Standard Sampling Curriculum.

The second level of QA/QC is an understanding of and compliance with these Standard Operating Procedures among everyone involved in the sampling event.

The third level of QA/QC is discrete procedures for analyzing quality using the data generated in the sampling program. These procedures are the responsibility of the Laboratory's internal QA program and the Steering Committee's Data Manager and Quality Assurance Officer.

A fourth and final level of QA/QC are activities at the statewide level by the Quality Assurance Committee that include:

- Review of QA/QC reports submitted with data by Steering Committees;
- Audits of Steering Committee QA/QC activities; and
- Comparison of data with statewide and nationwide databases;

The remainder of this section outlines QA/QC procedures that apply to Tier III and IV data uses.

b. Duplicate Samples and Field Blanks

The Sampling Event Coordinator selects sites for duplicate samples and field blanks in consultation with the Steering Committee's Science Advisor and Quality Assurance Officer. Sites selected for duplicate and blank samples should be chosen to be representative of the range of conditions encountered and to rotate through different sampling teams. Sites expected to be at or near method detection limits should be included. Duplicates should also be collected where high concentrations are expected.

1. Duplicate Samples

Watershed Watch uses concurrent duplicate samples to assess variability in sample collection, processing, and analysis.

Supervising Samplers for the sites selected by the random selection process receive a pre-marked duplicate sample container in addition to the pre-marked sample containers with instructions for taking and submitting a duplicate sample. All other procedures for samples are followed for Duplicate Samples.

2. Field blanks

Watershed Watch uses field blanks to assess for bias from contamination of the sample during any stage of sample collection, processing, and analysis.

Supervising Samplers for the sites selected by the random selection process receive a pre-marked field blank container in addition to the pre-marked sample container. The Supervising Sampler is instructed to obtain bottled water in a food store and pour the amount required for a sample into the container pre-marked for the field blank sample at the site and time the routine sample is taken. All other procedures for samples are followed for field blanks. An example of instructions to Supervising Samplers for taking field blanks may be found in Appendix G.

Watershed Watch Chain of Custody Record

Sample #	Stream Name	Date sample taken

Sampling Location (correct or add location info if necessary)	Time sample taken

Name of "Supervising Sampler" on site when sample collected:

Lab Notes:

If name not correct, please enter proper name in Comment Box		Sampler ID#
	Telephone:	

Flow Rate	48 Hr Rainfall "	Turbidity	Water Chemistry	
			Oxygen ppm	pH SU
☐ 0-Dry	☐ 0	☐ 0-Clear		
☐ 1-Ponded	☐ 0.1	☐ 1		
☐ 2-Low	☐ 0.5	☐ 2		
☐ 3-Normal	☐ 1.0	☐ 3-Turbid	Conductivity	Temp C
☐ 4-Bank Full	☐ 1.5			
☐ 5-Flood!	☐ 2.0 +			

General comments, questions, corrections, concerns or suggestions.

When transporting samples to the lab, it is necessary to have each person that controls the sample to sign when they receive it AND when they relinquish it.

Relinquished by:	Time/Date	Received by:	Time/Date

This form must accompany your sample to the lab. The first signature in the "relinquished by" column must match the "supervising Sampler's" name!

Make a copy for yourself, then send the original on its way with your sample runner. Please correct errors on the pre-printed part of this record. If you have questions or difficulties, please contact us at 1-800-928-0045 Ext 473

Total Phosphorus in Water

1. Discussion

MDL= 0.02 as of 5/2002

Principle

Separation into total dissolved and total recoverable forms of phosphorus depends on filtration of the water sample through a 0.45 μm membrane filter. Total recoverable phosphorus includes all phosphorus forms when the unfiltered, shaken sample is heated in the presence of sulfuric acid and ammonium peroxydisulfate. Total dissolved phosphorus includes all phosphorus forms when the filtered, shaken sample is heated in the presence of sulfuric acid and ammonium peroxydisulfate. Phosphorus is converted to orthophosphate by digesting the water sample with ammonium persulfate and diluted sulfuric acid. Ammonium molybdate and antimony potassium tartrate can then react in an acid medium with dilute solutions of orthophosphate to form an antimony-phosphate-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color intensity is proportional to the phosphorus concentration.

Sensitivity

The range of determination for this method is 0.05 mg/L to 1.00 mg/L P.

Interferences

Ferric iron must exceed 50 mg/L, copper 10 mg/L, or silica 10 mg/L, before causing an interference. Higher silica concentrations cause positive interferences over the range of the test, as follows: results are high by 0.005 mg/L of phosphorus for 20 mg/L of SiO2, 0.015 mg/L of phosphorus for 50 mg/L, and 0.025 mg/L of phosphorus for 100 mg/L. Because arsenic and phosphorus are analyzed similarly, arsenic can cause an interference if its concentration is higher than that of phosphorus.

Sample Handling and Preparation

Samples should be preserved only by refrigeration at 4 °C. A raw sample should be used in the analysis. The holding time for this analysis is 28 days.

2. Safety

Safety glasses, gloves, and a lab coat should be worn while performing this analysis due to the use of, and possible exposure to, strong acids and bases.

3. Apparatus

Varion 50 Spectroscopy system
Filtration Apparatus
- Coors 60242 Büchner funnels.
- Suction flasks, connected in series to a vacuum system.
- Reservoir for the filtrate, 500 mL.
- Trap which prevents liquid from entering the vacuum system, 1000 mL

Paper filters—7.5 cm, 1 μm. (VWR Cat. # 28321-005)
Analytical balance, capable of weighing to the nearest 0.0001 g.
Drying oven.
Desiccator.
Thermix Stirring Hot Plate—Model 610T

HCl Acid washed glassware—Refer to the **"Total P"** section of the Glassware GLP for further

details. Commercial detergents should never used. Glassware should be dedicated for Total P use only.

6 ½ oz. Disposable polystyrene specimen cups—Cups should be rinsed three times with DI water.

4. Reagents

Purity of Reagents—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society. Other grades may be used, provided it is first ascertained that the reagent is sufficiently high in purity to permit its use without lessening the accuracy of the determinations.

Purity of Water—Unless otherwise indicated, references to water shall be understood to mean Type I reagent grade water (Milli Q Water System) conforming to the requirements in ASTM Specification D1193.

Ammonium Peroxydisulfate—Place **20 g** of ammonium peroxydisulfate in a 50 mL volumetric flask. Dilute with water to volume. Add a magnetic stirrer to the flask and let the solution stir until all the crystals have dissolved (minimum of 20 minutes). Prepare daily.

(*enough for 30 beakers total*)

Solution Mixture—Dissolve 0.13 g of antimony potassium tartrate and 5.6 g of ammonium molybdate in approximately 700 mL of water. **Cautiously** add 70 mL of concentrated sulfuric acid. Allow the solution to cool and dilute to 1 liter. The solution must be kept in a polyethylene bottle away from heat. This solution is stable for one year.

Combined Reagent—Dissolve 0.50 g solid ascorbic acid in 100 mL of solution mixture. Prepare daily.

Phenolphthalein indicator solution—Dissolve 0.5 g of phenolphthalein in a mixture of 50 mL isopropyl alcohol and 50 mL water.

Sulfuric acid (31 + 69)—Slowly add 310 mL of concentrated H_2SO_4 to approximately 600 mL of water. Allow solution to cool and dilute to 1 liter.

Sodium Hydroxide, 10 N—Dissolve 400 g of NaOH in approximately 800 mL of water. Allow solution to cool and dilute to 1 liter.

Sodium Hydroxide, 1 N—Dissolve 40 g of NaOH in approximately 800 mL of water. Allow solution to cool and dilute to 1 liter.

Phosphorus stock solution (50 mg/L)—Dissolve **0.2197 g** of predried (105 °C for one hour) KH_2PO_4 in water and dilute to 1 liter. Prepare **daily**.

Phosphorus standard solution (2.5 mg/L)—Dilute 50 mL of the stock solution to exactly 1 liter of water. Prepare **daily**.

Blank—reagent grade water.

Total phosphorus stock QC solution—Using a commercially available Quality Control solution, dilute to desired range and record manufactures name, lot #, and date.

Quality control sample—Dilute total P stock solution so that QC value falls midway in analysis

working range (0.05-1.00 ppm). Using 6.11 ppm QC stock solution, dilute 25 mL of Total Phosphorous stock solution to 500 mL resulting in a concentration of 0.306 ppm.

Acid for glassware-Carefully add 250 mL of concentrated hydrochloric acid to approximately 600 ml of water. Dilute to 1 liter.

5. Procedure

A. Prepare the spectrophotometer by turning on the lamp and allowing it to warm up for at least one hour. See the Spectrophotometer GLP for a detailed listing of necessary computer commands.

B. Standards Prep

1. Prepare a series of phosphorus standards from the 2.5 mg/L phosphorus standard solution according to the following table. Dilute each to 50 mL with water.

Volume of phosphorus standard, mL	Standard concentration, ppm
1	0.05
2	0.10
4	0.20
7	0.35
10	0.50
15	0.75
20	1.00

2. Prepare all standards **daily**.

C. Sample Prep

1. Pour 50 mL of each of the two blanks, standards, samples, duplicates, and Total P QC's into 100 mL glass beakers. Add 3 - 6 glass boiling beads to each beaker.
2. Mark beakers at top of liquid with a Sharpie.
3. Add 1 mL of ammonium peroxydisulfate solution and 1 mL of H_2SO_4 (31+69) to each marked beaker.
4. Place beakers on the large hot plates that are located in the hood.
5. Turn the Temp. knob on the hot plates to "HI."
6. Let each sample (blank, standard, duplicate, or QC) stay on the hot plate until its volume decreases to 10 mL. This process takes approximately 1 to 1 ½ hours. Do **not** allow the samples to completely evaporate.
7. Allow each sample to cool in the hood.
8. Add a drop of phenolphthalein indicator solution to each sample.
9. Add 1 mL of 10 N NaOH to each sample.
10. Continue adjusting the pH's by adding 1 N NaOH until each sample becomes faint pink in color. The pH is approximately 10 at this point.
11. Bring samples back to colorless by adding 1 N H_2SO_4 to each sample. The pH is approximately 4 at this point.
12. Bring each sample's volume back up to the mark with water.
13. Filter each of the samples using the acid washed ceramic funnels and 1 µm paper filters.
14. Pour 25 mL of each sample into its corresponding 4 ½ oz. plastic beaker.
15. Add 5 mL of combined reagent to the sample and mix thoroughly.
16. After a minimum of 10 minutes, but no longer than 30 minutes, measure the absorbance of the blue color at **880** nm with the spectrophotometer.

D. Sample Analysis

1. The computer, by comparing the concentration of each calibration standard against its absorbance, can plot a calibration curve. The correlation coefficient must be $\geq$ 0.994 to be acceptable. If above criteria is not met the standards may need to be remade and rerun.
2. Once the spectrophotometer is standardized properly, the samples may be analyzed.
3. Once the analysis is completed, print out a copy of the standard values, plotted curve, and the sample values. Copy the relevant data onto the Total Phosphorous Data Sheet.

E. Clean Up

1. Turn off the spectrophotometer lamp.
2. The waste must be placed in the acid waste container.
3. For glassware clean up, refer to the **"Total P"** section of the Glassware GLP.

6. Quality Control

A quality control sample should be run at the beginning and end of each sample delivery group (SDG) or at the frequency of one per every ten samples. The QC's value should fall between ± 10 % of its theoretical concentration.

A duplicate analysis should be run for each SDG or at the frequency of one per every twenty samples, whichever is greater. The RPD (Relative Percent Difference) should be less than 10%. If this difference is exceeded, the duplicate must be reanalyzed.

From each pair of duplicate analytes (X_1 and X_2), calculate their RPD value:

$$\% \ RPD = 2 \bullet \left(\frac{X_1 - X_2}{X_1 + X_2} \right) x\, 100$$

where:

(X_1 - X_2) means the absolute difference between X_1 and X_2.

7. Method Performance

The method detection limit (MDL) should be established by determining seven replicates that are 2 to 5 times the instrument detection limit. The MDL is defined as the minimum concentration that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte.

$$MDL = t_{(n-1,1-\alpha\ =99)}(S)$$

where:

t = the t statistic for n number of replicates used (for n=7, t=3.143)
n = number of replicates
S = standard deviation of replicates

8. References

ASTM vol. 11.01 (1996), D 515, "Standard Test Methods for Phosphorus in Water", pg. 24

ASTM vol. 11.01 (1996), D 1193, " Specification for Water", pg. 116

EPA 365.2 Phosphorous , All Forms (Colorimetric, Ascorbic Acid)

Ion Chromatography of Water

1. Discussion

Principle

This method addresses the sequential determination of the following inorganic anions: *bromide, chloride, fluoride, nitrate, Kjeldahl nitrogen, total nitrogen* and *sulfate*. A small volume of water sample is injected into an ion chromatograph to flush and fill a constant volume sample loop. The sample is then injected into a stream of carbonate-bicarbonate eluent. The sample is pumped through three different ion exchange columns and into a conductivity detector. The first two columns, a precolumn (or guard column), and a separator column, are packed with low-capacity, strongly basic anion exchanger. Ions are separated into discrete bands based on their affinity for the exchange sites of the resin. The last column is a suppressor column that reduces the background conductivity of the eluent to a low or negligible level and converts the anions in the sample to their corresponding acids. The separated anions in their acid form are measured using an electrical conductivity cell. Anions are identified based on their retention times compared to known standards. Quantitation is accomplished by measuring the peak area and comparing it to a calibration curve generated from known standards.

Sensitivity

Ion Chromatography values for anions ranging from 0 to approximately 40 mg/L can be measured and greater concentrations of anions can be determined with the appropriate dilution of sample with deionized water to place the sample concentration within the working range of the calibration curve.

Interferences

Any species with retention time similar to that of the desired ion will interfere. Large quantities of ions eluting close to the ion of interest will also result in interference. Separation can be improved by adjusting the eluent concentration and /or flow rate. Sample dilution and/or the use of the method of Standard Additions can also be used. For example, high levels of organic acids may be present in industrial wastes, which may interfere with inorganic anion analysis. Two common species, formate and acetate, elute between fluoride and chloride. The water dip, or negative peak, that elutes near, and can interfere with, the fluoride peak can usually be eliminated by the addition of the equivalent of 1 mL of concentrated eluent (100X) to 100 mL of each standard and sample. Alternatively, 0.05 mL of 100X eluent can be added to 5 mL of each standard and sample.

Because bromide and nitrate elute very close together, they can potentially interfere with each other. It is advisable not to have Br^-/NO_3^- ratios higher than 1:10 or 10:1 if both anions are to be quantified. If nitrate is observed to be an interference with bromide, use of an alternate detector (e.g., electrochemical detector) is recommended.

Method Interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baseline in ion chromatograms. Samples that contain particles larger than 0.45 micrometers and reagent solutions that contain particles larger than 0.20 micrometers require filtration to prevent damage to instrument columns and flow systems. If a packed bed suppressor column is used, it will be slowly consumed during analysis and, therefore, will need to be regenerated. Use of either an anion fiber suppressor or an anion micro-membrane suppressor eliminates the time-consuming regeneration step by using a continuous flow of regenerant.

Because of the possibility of contamination, do not allow the nitrogen cylinder to run until it is empty. Once the regulator gauge reads 100 kPa, switch the cylinder out for a full one. The old cylinder should them be returned to room #19 for storage until the gas company can pick it up. Make sure that the status tag marks the cylinder as "EMPTY".

Sample Handling and Preservation

Samples should be collected in glass or plastic bottles that have been thoroughly cleaned and rinsed with reagent water. The volume collected should be sufficient to ensure a representative sample and allow for replicate analysis, if required. Most analytes have a 28 day holding time, with no preservative and cooled to $4^{o}C$. Nitrite, nitrate, and orthophosphate have a holding time of 48 hours. Combined nitrate/nitrite samples preserved with H_2SO_4 to a pH $\leq$2 can be held for 28 days; however, pH$\leq$2 and pH$\geq$12 can be harmful to the columns. It is recommended that the pH be adjusted to pH$\geq$2 and pH$\leq$12 just prior to analysis.

Note: Prior to analysis, the refrigerated samples should be allowed to equilibrate to room temperature for a stable analysis.

2. Apparatus

Dionex DX500
Dionex CD20 Conductivity Detector
Dionex GP50 Gradient Pump
Dionex Eluent Organizer
Dionex AS40 Automated Sampler
Dionex ASRS-Ultra Self-Regenerating Suppressor
Dionex Ionpac Guard Column (AG4A, AG9A, or AG14A)
Dionex Ionpac Analytical Column (AS4A, AS9A, or AS14A)
Dionex Chromeleon 6.8 Software Package
Dionex 5 mL Sample Polyvials and Filter Caps
2 L Regenerant Bottles
5 mL Adjustable Pipettor and Pipettor Tips
1 mL Adjustable Pipettor and Pipettor Tips
A Supply of Volumetric Flasks ranging in size from 25 mL to 2 L
A Supply of 45 micrometer pore size Cellulose Acetate Filtration Membranes
A Supply of 25x150 mm Test Tubes
Test Tube Racks for the above 25x150 mm Test Tubes
Gelman 47 mm Magnetic Vacuum Filter Funnel, 500 mL Vacuum Flask, and a Vacuum Supply

3. Reagents

Purity of Reagents—HPLC grade chemicals (where available) shall be used in all reagents for Ion Chromatography, due to the vulnerability of the resin in the columns to organic and trace metal contamination of active sites. The use of lesser purity chemicals will degrade the columns.

Purity of Water—Unless otherwise indicated, references to water shall be understood to mean Type I reagent grade water (Milli Q Water System) conforming to the requirements in ASTM Specification D1193.

Eluent Preparation for **SYSTEM2 NITRATE** *Methods, including Bromides (using AG4, AG4 and AS4 columns)*—All chemicals are predried at 105° C for 2 hrs then stored in the desiccator. Weigh out 0.191 g of sodium carbonate (Na_2CO_3) and 0.286 g of sodium bicarbonate ($NaHCO_3$) and dissolve in water. System 2 (the chromatography module that contains the AG4, AG4, and AS4 Dionex columns) to be sparged, using helium, of all dissolved gases before operation.

Eluent Preparation for **SYSTEM2 NITRATE** (F) *Method (using AG14 and AS14 columns)*—Weigh out 0.3696 g of sodium carbonate (Na_2CO_3) and 0.080 g of sodium bicarbonate (NaHCO3) and dissolve in water. Bring the volume to 1000 mL and place the eluent in the System 1 bottle marked for this eluent concentration. The eluent must be sparged using helium as in the above reagent for System 2.

Eluent Preparation for **SYSTEM2 TKN** (TKN) *Methods, including Total Nitrogen (using AG4A, AG4A, and AS4A columns)*—Weigh out 0.191 g of sodium carbonate (Na_2CO_3) and 0.143 g of sodium bicarbonate ($NaHCO_3$) and dissolve in water. Bring the volume up to 1000 ml and place in the System 2 bottle labeled "*IC-TKN 0.191/0.143*". Sparge the eluent as in the above reagent for System 2.

100X Sample Spiking Eluent—prepared by using the above carbonate/bicarbonate ratios, but increasing the concentration 100X. Weigh out 1.91 g of Na_2CO_3 and 2.86 g of $NaHCO_3$ into a 100 mL volumetric flask. 0.05 mL of this solution is added to 5 mL of all samples and standards to resolve the water dip associated with the fluoride peak.

Stock standard solutions, 1000 mg/L (1 mg/mL): Stock standard solutions may be purchased (SPEX) as certified solutions or prepared from ACS reagent grade materials (dried at 105^{o} C for 30 minutes

Calibration Standards—for the **SYSTEM2 NITRATE** (except Bromide) methods are prepared as follows:

1. *Calibration Standard 1:* Pipette 0.1 mL of 1000 mg/L $NaNO_3$ stock standard, 0.1 mL of 1000 mg/L NaF stock standard, 2 mL of 1000 mg/L NaCl stock standard, and 10 mL of 1000 mg/L K_2SO_4 stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.
2. *Calibration Standard 2*: Pipette 0.5 mL of 1000 mg/L $NaNO_3$ stock standard, 0.5 mL of 1000 mg/L NaF stock standard, 5 ml of 1000 mg/L NaCl stock standard, and 20 mL of 1000 mg/L K_2SO_4 stock standard into a 1000 mL volumetric flask, partially filled with water, then fill to volume.
3. *Calibration Standard 3*: Pipette 2.5 mL of 1000 mg/mL $NaNO_3$ stock standard, 2.5 mL of 1000 mg/L NaF stock standard, 10 mL of 1000 mg/L NaCl stock standard, and 40 mL of 1000 mg/L K_2SO_4 stock standard into a 1000 mL volumetric flask partially filled with deionized water, then fill to volume.
4. *Quality Control Sample:* Pipette 1.0 mL of 1000 mg/L $NaNO_3$ stock solution, 1.0 mL of 1000 mg/L NaF stock solution, 8 mL of 1000 mg/L NaCl stock solution, and 30 mL of mg/L K_2SO_4 stock standard into a 1000 mL volumetric flask, partially filled with water, then fill to volume.

Calibration Standards—for the **SYSTEM2 NITRATE** (Fluoride) method are prepared as follows:

1. *Calibration Standard 1*: Pipette 0.01 mL of 1000 mg/L NaF stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.
2. *Calibration Standard 2*: Pipette 0.05 mL of 1000 mg/L NaF stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.

3. *Calibration Standard 3*: Pipette 0.1 mL of 1000 mg/mL NaF stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.
4. *Calibration Standard 4*: Pipette 0.5 mL of 1000 µg/mL NaF stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.
5. *Calibration Standard 5*: Pipette 1.0 mL of 1000 mg/L 1000 stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.
6. *Quality Control Standard*: Pipette 0.1 mL of 1000 mg/L NaF from a separate source stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.
7. *Quality Control Standard*: Pipette 0.4 mL of 1000 mg/L NaF from a separate source stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.
8. *Quality Control Standard*: Pipette 1.0 mL of 1000 mg/L NaF from a separate source stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volum

Calibration Standards—for the **SYSTEM2 NITRATE** (Bromide) method are prepared as follows:

1. *Calibration Standard 1*: Pipette 2 mL of 1000 mg/L NaBr stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.
2. *Calibration Standard 2*: Pipette 5 mL of 1000 mg/L NaBr stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.
3. *Calibration Standard 3*: Pipette 10 mL of 1000 mg/L NaBr stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.
4. *Quality Control Standard*: Pipette 8 mL of 1000 mg/L NaBr stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.

Outside Source Certified Quality Control Sample—ERA

4. Procedure

A. Instrument Preparation

1. Before turning on the Dionex Ion Chromatography System:
   a. Fill the eluent reservoir(s) with fresh eluent.
   b. Make certain the waste reservoir is empty of all waste.
   c. Turn on the helium. The system pressure should be between 7 - 15psi. The system pressure can be regulated with the knob on the back of the Eluent Organizer.
   d. Connecting a piece of tubing to the gas line going into the eluent bottle and putting the tubing into the eluent degasses the eluent reservoir(s). The gas knob on the Eluent Organizer that corresponds to the eluent bottle should be slowly opened until a constant bubbling stream can be seen in the eluent bottle.
   e. The eluent should be degassed with helium, for a minimum of 30 minutes, before operation of the instrument.
   f. After the eluent has been degassed, remove the tube from the eluent and tightly seal the eluent bottle. The eluent is now ready to introduce into the system.
2. Whether using the IP25 for Fluorides or the GP50 for everything else, turn off the browser, scroll to **REMOTE** on the screen, select **LOCAL** and **ENTER**.
3. Scroll to mL/min., change to 0 mL/min., and hit **ENTER**. If using the IP25 pump, skip to step #5.
4. Hit **MENU** and select **1**, then **ENTER**.
5. Insert syringe into the Priming Block, open the gas valve on the Eluent Organizer, turn the valve on the Priming Block counterclockwise, and turn on the pump that corresponds with the method to be ran by pushing the **OFF/ON** button.
6. If the syringe does not fill freely, assist by gently pulling back on the plunger of the syringe. Make certain that all of the air bubbles are removed from the eluent line to the pumps.
7. Press **OFF/ON** on the pump to turn it off.

8. Turn the valve on the Priming Block clockwise, remove the syringe and expel the air bubbles from the syringe.
9. Reinsert the syringe filled with eluent into the Priming Block.
10. Open the valve on the Pressure Transducer and the valve on the Priming Block with the eluent filled syringe still attached. This is accomplished by turning both counterclockwise.
11. Press **PRIME** on the pump and push the contents of the syringe into the Priming Block. After the eluent has been injected into the Priming Block, press **OFF/ON** to turn the prime pump off and to close the valves on the Pressure Transducer and Priming Block.
12. Remove the syringe from the Priming Block.
13. Scroll to the mL/min. on the screen for the pump. For the GP50, type 2 mL/min., and press **ENTER**. For the IP25, type 1.2 mL/min., and press **ENTER**.
14. Press **OFF/ON** to turn on the pump at the appropriate rate. The pressure should soon stabilize between both pumpheads after two minutes of pumping time.
15. If the pressure between pumpheads has a difference >20 psi, then shut down the pump and repeat steps 2-14 to remove air bubbles and prime the pumps.
16. Once the pump has a pumping pressure difference between pumpheads of <20 psi, then go to the computer and enter PeakNet.
17. On the computer**, turn on the Chromeleon 6.8 browser**, then choose either **System 1** (Fluoride) or **System 2** (all other anions including Bromide and TKN).
18. Go to **last run sequence, click to highlight and go to file, click save as.**. This will load the method of interest and a template for the current sequence run.
19. The sequence is edited to reflect the method and samples that are to be run.
    a. **SYSTEM2 NITRATE** for Fluoride
    b. **SYSTEM2 NITRATE** for Bromides
    c. **SYSTEM2 TKN** for TKN and Total Nitrogen

Note: Data is reprocessed in the section of Chromelon 6.8 called *Sequence integration editor.* Only operators with a <u>minimum of three months</u> experience in Ion Chromatography should attempt to reprocess data for this analysis. Once data is optimized, then the nitrogen values from nitrate and nitrite analysis can be subtracted from this value for the TKN nitrogen value. If only Total Nitrogen is needed then use the optimized data value without the correction for nitrite and nitrate nitrogen.

    d. **SYSTEM 2 NITRATE** for all other anions,

20. Observe the reading on the screen of the CD20 Conductivity Detector. A conductivity rate change of <0.03 μS over a 30 second time span is considered stable for analysis.
21. If using the GP50 pump, it will take about 15-30 minutes for the CD20 system to stabilize. If using the IP25, it will take between 30 minutes to 2 hours for stabilization.
22. Once the CD20 is stabilized, the Dionex DX500 Ion Chromatography System is ready to start standardization.

NOTE: When using the GP50 Gradient Pump, all due care must be taken before one switches from local procedures to remote procedures. The bottle from which the eluent is being pumped (i.e., A, B, C, or D) must exactly match the bottle specified in the method. If there is a difference, then once the pump control is turned over to remote control, irreversible damage and destruction of suppressors, columns, piston seals, and check valves on the GP50 Gradient Pump will occur. <u>NEVER</u> switch from bottle C to A, B, or D without flushing the system lines with water to remove all traces of eluent from bottle C from the lines.

B. <u>Sample Preparation</u>

1. If the sample was not filtered in the field, it must be done so now. Transfer 50 mL of a well-mixed sample to the filtering apparatus. Apply the suction and collect the filtrate.

2. If the conductivity values for the sample are high, dilution will be necessary to properly run the sample within the calibration standard range. Dilutions are made in the Polyvials with the plastic Filter Caps. If the dilutions are > 20X, then volumetric glassware is required.
3. All dilutions are performed with reagent grade DI water. Be sure to mix the dilution well.
4. For Fluorides and Bromides, pipette 5.0 mL of the filtered samples into the Polyvials. For all other anions, including TKN and Total Nitrogen, first pipette 0.05 mL of 100X sample spiking eluent into the Polyvials, then pipette 4.95 mL of the filtered samples on top of the spiking eluent.
5. The Filter Caps are pressed into the Polyvials using the insertion tool.
6. Place the Polyvials into the Sample Cassette, which is placed into the Autosampler.
7. The white/black dot on the Sample Cassette should be located on right-hand side when loaded in the left-hand side of the Automated Sampler for System 2.
8. For every ten samples the following should be included:
   a. 1 DI water blank
   b. 1 Duplicate of any one sample
   c. 1 Quality Control sample/calibration check

C. Calibration and Sample Analysis

1. Set up the instrument with proper operating parameters established in the operation condition procedure
2. The instrument must be allowed to become thermally stable before proceeding. This usually takes 1 hour from the point on initial degassing to the stabilization of the baseline conductivity.
3. To run samples on the Dionex Ion Chromatography System:
   a. Make a run schedule on the Chromeleon 6.8 Software Section labeled **SEQUENCE**.
   b. Double click the mouse on the **SYSTEM 1 SEQUENCES or SYSYTEM 2 SEQUENCES** to display the Scheduler Area. The name of the calibration standards must be entered under the sample name section as **Standard #1, Standard #2**, and **Standard #3**.

   Note: Level must be changed to the corresponding standard level or the calibration will be in error. (Example: Standard #1 = Level #1; Standard #5 = Level #5)

   c. Next, enter QC, blanks, QC, samples, duplicates, QC, and blanks, in that order.
   d. Under sample type, click on either **Calibration Standard** or **Sample**, depending on what is being run.
   e. Under the **Method** section, the method name must be entered. To do so, double click on the highlighted area under **Method**, scroll through the list of methods and double click on the method of interest.
   f. Next under the **Data File** section, enter the name of the data file.
   g. Finally, in the **Dil** area, type in the dilution factor if different from 1. Do this for all standards, blanks, quality controls, duplicates, and samples to be run under this schedule.
   h. Save the schedule and obtain a printout of it.
   i. Standardize the Dionex Ion Chromatography System by running the standards: **Standard #1**, **Standard #2**, and **Standard #3**.
4. Run the QC standards.
5. Run the prepblank and DI water blank.
6. Run the samples, duplicates, and blanks.
7. Run the QC standards at the end.

5. Calculations

A. Calculations are based upon the ratio of the peak area and concentration of standards to the peak area for the unknown. Peaks at the same or approximately the same retention times are compared. Once the method has been updated with the current calibration, this is calculated automatically by the software using linear regression. Remember that when dilutions are being run, the correct dilution factor must be entered.

B. Manual calculations are based upon the ratio of the peak and concentration of standards to the peak area for the unknown when the software will not automatically calculate the unknown concentration. Peaks at the same or approximately the same retention times are compared. The unknown concentration can be calculated from using this ratio. Remember that when dilutions are being run that the correct dilution factor must be entered before you will get the correct result.

C. When possible the unknown should be bracketed between two knowns and the calculation of the unknown made from both for comparison.

6. Quality Control

A quality control sample obtained from an outside source must first be used for the initial verification of the calibration standards. A fresh portion of this sample should be analyzed every week to monitor stability. If the results are not within +/- 10 % of the true value listed for the control sample, prepare a new calibration standard and recalibrate the instrument. If this does not correct the problem, prepare a new standard and repeat the calibration. A quality control sample should be run at the beginning and end of each sample delivery group (SDG) or at the frequency of one per every ten samples. The QC's value should fall between ± 10 % of its theoretical concentration.

A duplicate should be run for each SDG or at the frequency of one per every twenty samples, whichever is greater. The RPD (Relative Percent Difference) should be less than 10%. If this difference is exceeded, the duplicate must be reanalyzed.

From each pair of duplicate analytes (X_1 and X_2), calculate their RPD value:

$$\% \; RPD = 2 \bullet \left(\frac{X_1 - X_2}{X_1 + X_2} \right) x \, 100$$

where:

(X_1 - X_2) means the absolute difference between X_1 and X_2.

7. Method Performance

The method detection limit (MDL) should be established by determining seven replicates that are 2 to 5 times the instrument detection limit. The MDL is defined as the minimum concentration that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte.

$$MDL = t_{(n-1,1-\alpha\ =99)}(S)$$

where:

t = the t statistic for n number of replicates used (for n=7, t=3.143)
n = number of replicates
S = standard deviation of replicates

8. Reference

EPA SW 846-9056, Chapter 5, September 1994

U.S. EPA Method 300.0, March 1984

ASTM vol. 11.01 (1996), D 4327, “Standard Test Method for Anions in Water by Chemically
Suppressed Ion Chromatography”.

0/2010 addendum to 01/2009 Ion Chromatography of Water

1. Discussion

Principle

and iodine.

3. Reagents

Calibration Standards

1. Calibration Standard 1: Pipette 0.1 mL of 1000 mg/L I stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.
2. Calibration Standard 2: Pipette 0.5 mL of 1000 mg/L I stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.
3. Calibration Standard 3: Pipette 1.0 mL of 1000 mg/L I stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.
4. Calibration Standard 4: Pipette 5.0 mL of 1000 mg/L I stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.
5. Calibration Standard 5: Pipette 10.0 mL of 1000 mg/L I stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.
6. Quality Control Sample: Pipette 5.0 mL of 1000 mg/L I stock standard into a 1000 mL volumetric flask partially filled with water, then fill to volume.

Orthophosphate as Phosphate in Water

1. Discussion

MDL 0.003 as of 5/2002

MDL 0.009 as PO4

Principle

Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of orthophosphate to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the orthophosphate concentration.

Sensitivity

The range of determination for this method is 0.010 mg/L to 1.000 mg/L (in the PO_4–P form), when analyzed at 880.0 nm. (Method states using 650nm wavelength or 880.0nm if instrument is capable and the Cary 50 UV-Vis is capable).

Interferences

Arsenate, iron and silica cause interferences. Reducing the arsenic acid to arsenious acid with sodium bisulfite may eliminate arsenate interference. When high concentrations of iron are present, recovery of phosphorus will be lowered because the iron will use some of the reducing agent. The bisulfite treatment will also eliminate this interference. High silica concentrations cause positive interference.

Sample Handling and Preparation

If possible, a filtered aliquot should be used. If the analysis cannot be performed the day of collection, the sample should be preserved by the addition of 2 ml concentrated H_2SO_4 per liter and refrigerated at 4 °C. Recommended holding time is 48 hours. Suspended solids should be removed by filtration.

Note: Samples should be filtered PRIOR to preservation.

2. Safety

Safety glasses, gloves, and a lab coat should be worn while performing this analysis due to the use of and possible exposure to strong acids.

3. Apparatus

Varion 50 Spectroscopy system

Filtration Apparatus:

- Gelman 47mm magnetic filter funnel.
- Suction flasks, connected in series to a vacuum system.
- Reservoir for the filtrate, 500 mL.
- Trap which prevents liquid from entering the vacuum system, 1000 mL.

Cellulose-acetate filters—Micron Separations, Inc. 47 mm, 0.45 micron cellulose acetate filter membrane.

Analytical balance—capable of weighing to the nearest 0.0001 g.

Drying oven.

Desiccator.

4 1/2 oz. plastic beakers—Must be single-use only. Rinse three times with DI water.

4. Reagents

Purity of Reagents—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society. Other grades may be used, provided it is first ascertained that the reagent is sufficiently high in purity to permit its use without lessening the accuracy of the determinations.

Purity of water—Unless otherwise indicated, references to water shall be understood to mean Type 1 reagent grade water (Milli Q Water System) conforming to the requirements in ASTM Specification D1193.

Ammonium molybdate-antimony potassium tartrate solution—This solution has a long shelf life and is stable for approximately **six months**. It is stored in a one liter plastic bottle. Dissolve 8 g of ammonium molybdate and 0.2 g antimony potassium tartrate in 800 mL water and dilute to 1 liter.

Ascorbic acid solution—This solution has a shelf life of **two weeks** and should be stored in a one liter amber bottle (in the refrigerator). Be sure to indicate on the label when the solution was made. Dissolve 15 g of ascorbic acid in 125 mL water and dilute to 250 mL. Add 0.5 mL of spec. grade acetone.

Sulfuric acid, 12 N—This solution is stable for up to **six months**. It is stored in a one liter plastic bottle. Slowly add 333.3 ml concentrated H_2SO_4 to approximately 600 mLwater. Allow solution to cool and dilute to 1000 mL.

Phosphate as P Stock (1000ppm)--Purchases stock from ERA

Phosphate as P Standard Solution (10mg/L)—5mls of 1000mg/L stock in 500ml flask dilute with Milli-Q water

Quality control sample—Dilute ortho-p QC stock solution so that the QC value falls within analysis working range (0.01-1.00). QC is purchased from ERA.

Ortho-P QC stock solution—Using a commercially available Quality Control solution, dilute to desired range and record manufacturers name, lot #, and date.

Blank—Reagent Grade DI Water

5. Procedure

A. Prepare the spectrophotometer by turning on the lamp and allowing it to warm up for at least one hour. See the Spectrophotometer GLP for a detailed listing of necessary computer commands.

B. Standards Prep

1. Prepare a series of phosphorus standards from the phosphorus standard solution (10 mg/L) according to the following table-dilute to volume with water.

Volume of standard, mL	Standard concentration, ppm	Final Volume, mL
0.5	0.02	250
1	0.05	200

5	0.10	500
5	0.20	250
10	0.50	200
20	0.80	250
50.0	1.00	500

2. These standards must be prepared **daily**.

B. Sample Prep

1. If the samples were not filtered in the field, follow below procedure to do so now.
   a. Begin by rinsing all filtering apparatuses with water (magnetic filter funnel, magnetic filter membrane, and suction flask).
   b. Place the magnetic filter membrane tightly on the suction flask and turn on the vacuum. This will remove any water remaining in the filter funnel. After a few seconds, turn off the vacuum pump.
   c. Using small tweezers, place a cellulose-acetate filter on each of the magnetic filter membranes and turn on the vacuum pump. Place a magnetic filter funnel on top of each magnetic filter membrane.
   d. Pour a small amount of sample (10 mL or less) into the funnel and let it collect in the suction flask. When the entire sample has drained through, stop suction; disconnect the suction hose from one flask at the plastic connector junction.
   e. Remove both the magnetic funnel and the magnetic filter membrane together (do not disturb the filter) and carefully lower a 50 mL glass collection tube into the suction flask. Reconnect the apparatus and filter the sample into the collection tube.
   f. Once filtered, measure out 50 mL of sample and transfer it into a 100 mL plastic beaker (measure using a graduated cylinder). This may require two separate filterings.
2. Add 1 mL of 12 N H_2SO_4 and 4 mL of ammonium molybdate-antimony potassium tartrate to all standards and sample samples. Swirl to mix thoroughly.
3. Add 2 mL of ascorbic acid solution to all standards and samples. Swirl to mix thoroughly.
4. After waiting 5 minutes, the samples, standards, and QC's are ready to be analyzed at **880 nm**. The blue color is stable for one hour.

C. Sample Analysis

1. Place each standard and / or sample under probe submersing probe in solution swirl gently tap probe(releasing bubbles) and read or pour into cuvet and read.
2. The computer, by comparing the concentration of each calibration standard against its absorbance, can plot a calibration curve. The correlation coefficient must be $\geq$ 0.994 to be acceptable. If above criteria is not met, the standards may need to be remade and rerun.
3. Once the spectrophotometer is standardized properly, the samples may be analyzed.
4. When completed, print out a copy of the standard values, plotted curve, and the sample values. Copy the relevant data onto the Orthophosphate Data Sheet.

D. Clean Up

1. Turn off the spectrophotometer lamp.
2. Pour waste in acidic waste container.
3. For glassware clean up, refer to the **"NORMAL"** section of the Glassware GLP.

6. Calculations

The value read from the spectrophotometer is in the form **PO_4-P**. This value may need to be converted to the PO_4 form. The equation for this conversion is: **PO_4- P \ 0.32614 = PO_4**.

7. Quality Control

A calibration curve should be established using the standards described in section 3. Each point on this curve must be within ± 10 % error or the calibration curve must have a r^2 value of 0.994 or above. Any point that is not within this range or that causes a low r^2 value must be redeveloped and rerun until either above criteria are satisfied.

The quality control sample should be run at the beginning and end of each sample delivery group (SDG) or at the frequency of one per every ten samples. The QC's value should fall between ± 10 % of its theoretical concentration.

A duplicate should be run for of each SDG or at the frequency of one per every twenty samples, whichever is greater. The RPD (Relative Percent Difference) should be less than 10%. If this difference is exceeded, the sample must be reanalyzed.

From each pair of duplicate analytes (X_1 and X_2), calculate their RPD value:

$$\% \, RPD = 2 \bullet \left(\frac{X_1 - X_2}{X_1 + X_2} \right) x \, 100$$

where:

(X_1 - X_2) means the absolute difference between X_1 and X_2.

If a sample's value exceeds 1.000 ppm, the sample must be diluted. The sample must be diluted so that its concentration falls between 0.010 ppm and 1.000 ppm. The sample must be diluted using volumetric flasks and pipettes.

8. Method Performance

The method detection limit (MDL) should be established by determining seven replicates that are 2 to 5 times the instrument detection limit. The MDL is defined as the minimum concentration that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte.

$$MDL = t_{(n-1,1-\alpha=99)}(S)$$

where:

t = the t statistic for n number of replicates used
n = number of replicates
S = standard deviation of replicates

9. References

U.S. EPA Method 365.3, 1978

Standard Methods for the Examination of Water and Wastewater, 20th edition (1998), Method 4500-P E, pg. 4-146

TKN & Total Nitrogen by Ion Chromatography of Water

1. Discussion

Principle

This method addresses the sequential determination of the following inorganic anions: *bromide, chloride, fluoride, nitrate, Kjeldahl nitrogen, total nitrogen* and *sulfate*. A small volume of water sample is injected into an ion chromatograph to flush and fill a constant volume sample loop. The sample is then injected into a stream of carbonate-bicarbonate eluent. The sample is pumped through three different ion exchange columns and into a conductivity detector. The first two columns, a precolumn (or guard column), and a separator column, are packed with low-capacity, strongly basic anion exchanger. Ions are separated into discrete bands based on their affinity for the exchange sites of the resin. The last column is a suppressor column that reduces the background conductivity of the eluent to a low or negligible level and converts the anions in the sample to their corresponding acids. The separated anions in their acid form are measured using an electrical conductivity cell. Anions are identified based on their retention times compared to known standards. Quantitation is accomplished by measuring the peak area and comparing it to a calibration curve generated from known standards.

Sensitivity

Ion Chromatography values for anions ranging from 0 to approximately 40 mg/L can be measured and greater concentrations of anions can be determined with the appropriate dilution of sample with deionized water to place the sample concentration within the working range of the calibration curve.

Interferences

Any species with retention time similar to that of the desired ion will interfere. Large quantities of ions eluting close to the ion of interest will also result in interference. Separation can be improved by adjusting the eluent concentration and /or flow rate. Sample dilution and/or the use of the method of Standard Additions can also be used. For example, high levels of organic acids may be present in industrial wastes, which may interfere with inorganic anion analysis. Two common species, formate and acetate, elute between fluoride and chloride. The water dip, or negative peak, that elutes near, and can interfere with, the fluoride peak can usually be eliminated by the addition of the equivalent of 1 mL of concentrated eluent (100X) to 100 mL of each standard and sample. Alternatively, 0.05 mL of 100X eluent can be added to 5 mL of each standard and sample.

Because bromide and nitrate elute very close together, they can potentially interfere with each other. It is advisable not to have Br^-/NO_3^- ratios higher than 1:10 or 10:1 if both anions are to be quantified. If nitrate is observed to be an interference with bromide, use of an alternate detector (e.g., electrochemical detector) is recommended.

Method Interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baseline in ion chromatograms. Samples that contain particles larger than 0.45 micrometers and reagent solutions that contain particles larger than 0.20 micrometers require filtration to prevent damage to instrument columns and flow systems. If a packed bed suppressor column is used, it will be slowly consumed during analysis and, therefore, will need to be regenerated. Use of either an anion fiber suppressor or an anion micro-membrane suppressor eliminates the time-consuming regeneration step by using a continuous flow of regenerant.

Because of the possibility of contamination, do not allow the nitrogen cylinder to run until it is empty. Once the regulator gauge reads 100 kPa, switch the cylinder out for a full one. The old cylinder should them be returned to room #19 for storage until the gas company can pick it up. Make sure that the status tag marks the cylinder as "EMPTY".

Sample Handling and Preservation
Samples should be collected in glass or plastic bottles that have been thoroughly cleaned and rinsed with reagent water. The volume collected should be sufficient to ensure a representative sample and allow for replicate analysis, if required. Most analytes have a 28 day holding time, with no preservative and cooled to 4°C. Nitrite, nitrate, and orthophosphate have a holding time of 48 hours. Combined nitrate/nitrite samples preserved with H_2SO_4 to a pH $\leq$2 can be held for 28 days; however, pH$\leq$2 and pH$\geq$12 can be harmful to the columns. It is recommended that the pH be adjusted to pH$\geq$2 and pH$\leq$12 just prior to analysis. Preserved samples should not be used for TKN and Total Nitrogen analysis.

Note: Prior to analysis, the refrigerated samples should be allowed to equilibrate to room temperature for a stable analysis.

2. Apparatus

Dionex DX500
Dionex CD20 Conductivity Detector
Dionex GP50 Gradient Pump
Dionex Eluent Organizer
Dionex AS40 Automated Sampler
Dionex ASRS-Ultra Self-Regenerating Suppressor
Dionex Ionpac Guard Column (AG4A)
Dionex Ionpac Analytical Column (AS4A)
Dionex Chromeleon 6.8 Software Package
Dionex 5 mL Sample Polyvials and Filter Caps
2 L Regenerant Bottles
5 mL Adjustable Pipettor and Pipettor Tips
1 mL Adjustable Pipettor and Pipettor Tips
A Supply of Volumetric Flasks ranging in size from 25 mL to 2 L
A Supply of 45 micrometer pore size Cellulose Acetate Filtration Membranes
A Supply of 25x150 mm Test Tubes
Test Tube Racks for the above 25x150 mm Test Tubes
Gelman 47 mm Magnetic Vacuum Filter Funnel, 500 mL Vacuum Flask, and a Vacuum Supply

3. Reagents

Purity of Reagents—HPLC grade chemicals (where available) shall be used in all reagents for Ion Chromatography, due to the vulnerability of the resin in the columns to organic and trace metal contamination of active sites. The use of lesser purity chemicals will degrade the columns.

Purity of Water—Unless otherwise indicated, references to water shall be understood to mean Type I reagent grade water (Milli Q Water System) conforming to the requirements in ASTM Specification D1193.

AG4A, and AS4A columns)—Weigh out 0.191 g of sodium carbonate (Na_2CO_3) and 0.143 g of sodium bicarbonate ($NaHCO_3$) and dissolve in water. Bring the volume up to 1000 ml and place in the System 2 bottle labeled "*IC-TKN 0.191/0.143*". Sparge the eluent as in the above reagent for System 2.

100X Sample Spiking Eluent—prepared by using the above carbonate/bicarbonate ratios, but increasing the concentration 100X. Weigh out 1.91 g of Na_2CO_3 and 1.43 g of $NaHCO_3$ into a 100 mL volumetric flask. 0.05 mL of this solution is added to 5 mL of all samples and standards to resolve the water dip associated with the fluoride peak.

Borate Buffer Solution---Dissolve 61.8 g H_3BO_3 and 8.0 g NaOH in a 1L volumetric flask containing at least 500 mL of DI water. Swirl to mix and bring to volume. Make fresh every 3 months.

Digestion Reagent---Dissolve 20.1 g of K_2SO_8 and 3.0 g of NaOH in a 1L flask containing at least 500 mL of DI water. Swirl to mix and bring to volume. Make fresh every 3 months.

Quality Control---Commercially available wastewater TKN standard (Environmental Resource Associates, "Ready-To-Use Wastewater QC Standards", Cat # 743, Arvada, CO, 1-800-ERA-0122)

Glutamic Acid Stock Standard ($C_3H_5NH_2(COOH)_2$), 100 ppm---Dry Glutamic Acid in oven at 105^0C for 24 hours. Cover and place in dissector until cool. Dissolve 1.051g in DI water and dilute to 1L; preserve with 2mL chloroform ($CHCl_3$). Store in refrigerator for no longer than 6 months.

Stock standard solutions, 1000 mg/L (1 mg/mL): Stock standard solutions may be purchased (SPEX) as certified solutions or prepared from ACS reagent grade materials (dried at 105^o C for 24 hours.

Nitrate Stock Standard (NO_3-N), 1000 ppm--- dry Potassium Nitrate (KNO_3) in oven , cover and place in dissector until cool. Dissolve 0.7218g in DI water and bring to 1 L; preserve with 2 mL chloroform ($CHCl_3$).

Nitrate Working Standard, 10 ppm---Dilute 100 mL of Nitrate Stock Standard to 1000 mL in 1L flask. Preserve with 2 mL chloroform ($CHCl_3$). Store in refrigerator for no longer than 6 months.

Calibration Standards—for the **SYSTEM 2 TKN** methods are prepared as follows:

1. *Using the 100 ppm Glutamic Acid Stock Standard, prepare the following:*
   a. 0.4 ppm = 1 mL of 100 ppm diluted to 250 mL
   b. 0.8 ppm = 2 mL of 100 ppm diluted to 250 mL
   c. 1.6 ppm = 4 mL of 100 ppm diluted to 250 mLl
2. *Using the 10 ppm Nitrate Stock Standard, prepare the following:*
   a. 0.1 ppm = 1 mL of 10 ppm diluted to 100 mL
   b. 0.2 ppm = 2 mL of 10 ppm diluted to 100 mL
   c. 0.4 ppm = 4 mL of 10 ppm diluted to 100 mL
   d. 0.8 ppm = 8 mL of 10 ppm diluted to 100 mL
   e. 1.6 ppm = 16 mL of 10 ppm diluted to 100 mL
   f. 2.9 ppm = 29 mL of 10 ppm diluted to 100 mL
3. *The QC is diluted from the ordered solution:* perform an appropriate dilution creating a QC with a value on calibration curve, (~1.5 ppm) using the ordered standard.

4. If it is deemed necessary, ICV's (Initial Calibration Verification) and CCV's (continuing Calibration Verification) can be run using a 0.8 ppm and/or 1.6 ppm glutamic acid solution.

Outside Source Certified Quality Control Sample—ERA

4. Procedure

A. Instrument Preparation

1. Before turning on the Dionex Ion Chromatography System:
   a. Fill the eluent reservoir(s) with fresh eluent.
   b. Make certain the waste reservoir is empty of all waste.
   c. Turn on the helium. The system pressure should be between 7 - 15psi. The system pressure can be regulated with the knob on the back of the Eluent Organizer.
   d. Connecting a piece of tubing to the gas line going into the eluent bottle and putting the tubing into the eluent degasses the eluent reservoir(s). The gas knob on the Eluent Organizer that corresponds to the eluent bottle should be slowly opened until a constant bubbling stream can be seen in the eluent bottle.
   e. The eluent should be degassed with helium, for a minimum of 30 minutes, before operation of the instrument.
   f. After the eluent has been degassed, remove the tube from the eluent and tightly seal the eluent bottle. The eluent is now ready to introduce into the system.
2. Whether using the IP25 for Fluorides or the GP50 for everything else, turn off the browser, scroll to **REMOTE** on the screen, select **LOCAL** and **ENTER**.
3. Scroll to mL/min., change to 0 mL/min., and hit **ENTER**. If using the IP25 pump, skip to step #5.
4. Hit **MENU** and select **1**, then **ENTER**.
5. Insert syringe into the Priming Block, open the gas valve on the Eluent Organizer, turn the valve on the Priming Block counterclockwise, and turn on the pump that corresponds with the method to be ran by pushing the **OFF/ON** button.
6. If the syringe does not fill freely, assist by gently pulling back on the plunger of the syringe. Make certain that all of the air bubbles are removed from the eluent line to the pumps.
7. Press **OFF/ON** on the pump to turn it off.
8. Turn the valve on the Priming Block clockwise, remove the syringe and expel the air bubbles from the syringe.
9. Reinsert the syringe filled with eluent into the Priming Block.
10. Open the valve on the Pressure Transducer and the valve on the Priming Block with the eluent filled syringe still attached. This is accomplished by turning both counterclockwise.
11. Press **PRIME** on the pump and push the contents of the syringe into the Priming Block. After the eluent has been injected into the Priming Block, press **OFF/ON** to turn the prime pump off and to close the valves on the Pressure Transducer and Priming Block.
12. Remove the syringe from the Priming Block.
13. Scroll to the mL/min. on the screen for the pump. For the GP50, type 2 mL/min., and press **ENTER**. For the IP25, type 1.2 mL/min., and press **ENTER**.
14. Press **OFF/ON** to turn on the pump at the appropriate rate. The pressure should soon stabilize between both pumpheads after two minutes of pumping time.
15. If the pressure between pumpheads has a difference >20 psi, then shut down the pump and repeat steps 2-14 to remove air bubbles and prime the pumps.
16. Once the pump has a pumping pressure difference between pumpheads of <20 psi, then go to the computer and enter PeakNet.

17. On the computer**, turn on the Chromeleon 6.8 browser**, then choose **System 2** (all other anions including Bromide and TKN).
18. Go to **last run sequence, click to highlight and go to file, click save as.**. This will load the method of interest and a template for the current sequence run.
19. The sequence is edited to reflect the method and samples that are to be run.
    a. **SYSTEM 2 TKN** for TKN and Total Nitrogen

Note: Data is reprocessed in the section of Chromeleon 6.8 called *Sequence integration editor.* Only operators with a <u>minimum of three months</u> experience in Ion Chromatography should attempt to reprocess data for this analysis. Once data is optimized, then the nitrogen values from nitrate and nitrite analysis can be subtracted from this value for the TKN nitrogen value. If only Total Nitrogen is needed then use the optimized data value without the correction for nitrite and nitrate nitrogen.

20. Observe the reading on the screen of the CD20 Conductivity Detector. A conductivity rate change of <0.03 μS over a 30 second time span is considered stable for analysis.
21. If using the GP50 pump, it will take about 15-30 minutes for the CD20 system to stabilize. If using the IP25, it will take between 30 minutes to 2 hours for stabilization.
22. Once the CD20 is stabilized, the Dionex DX500 Ion Chromatography System is ready to start standardization.

NOTE: When using the GP50 Gradient Pump, all due care must be taken before one switches from local procedures to remote procedures. The bottle from which the eluent is being pumped (i.e., A, B, C, or D) must exactly match the bottle specified in the method. If there is a difference, then once the pump control is turned over to remote control, irreversible damage and destruction of suppressors, columns, piston seals, and check valves on the GP50 Gradient Pump will occur. <u>NEVER</u> switch from bottle C to A, B, or D without flushing the system lines with water to remove all traces of eluent from bottle C from the lines.

B. <u>Sample Preparation</u>

1. If the sample was not filtered in the field, it must be done so now. Transfer 50 mL of a well-mixed sample to the filtering apparatus. Apply the suction and collect the filtrate.

2. If the conductivity values for the sample are high, dilution will be necessary to properly run the sample within the calibration standard range. Dilutions are made in the Polyvials with the plastic Filter Caps. If the dilutions are > 20X, then volumetric glassware is required.
3. All dilutions are performed with reagent grade DI water. Be sure to mix the dilution well.
4. For the anions TKN and Total Nitrogen, first pipette 0.05 mL of 100X sample spiking eluent into the Polyvials, then pipette 4.95 mL of the filtered samples on top of the spiking eluent.
5. The Filter Caps are pressed into the Polyvials using the insertion tool.
6. Place the Polyvials into the Sample Cassette, which is placed into the Autosampler.
7. The white/black dot on the Sample Cassette should be located on right-hand side when loaded in the left-hand side of the Automated Sampler for System 2.
8. For every ten samples the following should be included:
    a. 1 DI water blank
    b. 1 Duplicate of any one sample
    c. 1 Quality Control sample/calibration check

C. <u>Calibration and Sample Analysis</u>

1. Set up the instrument with proper operating parameters established in the operation condition procedure
2. The instrument must be allowed to become thermally stable before proceeding. This usually takes 1 hour from the point on initial degassing to the stabilization of the baseline conductivity.
3. To run samples on the Dionex Ion Chromatography System:
   a. Make a run schedule on the PeakNet Software Section labeled **SEQUENCE**.
   b. Double click the mouse on the **SYS 2** to display the Scheduler Area.
   The name of the calibration standards must be entered under the sample name section
   as **Standard #1, Standard #2**, and **Standard #3**.

   Note: Level must be changed to the corresponding standard level or the calibration will be in error. (Example: Standard #1 = Level #1; Standard #5 = Level #5)

   c. Next, enter QC, blanks, QC, samples, duplicates, QC, and blanks, in that order.
   d. Under sample type, click on either **Calibration Standard** or **Sample**, depending on what is being run.
   e. Under the **Method** section, the method name must be entered. To do so, double click on the highlighted area under **Method**, scroll through the list of methods and double click on the method of interest.
   f. Next under the **Data File** section, enter the name of the data file.
   g. Finally, in the **Dil** area, type in the dilution factor if different from 1. Do this for all standards, blanks, quality controls, duplicates, and samples to be run under this schedule.
   h. Save the schedule and obtain a printout of it.
   i. Standardize the Dionex Ion Chromatography System by running the standards: **Standard #1**, **Standard #2**, and **Standard #3**.
4. Run the QC standards.
5. Run the prepblank and DI water blank.
6. Run the samples, duplicates, and blanks.
7. Run the QC standards at the end.

5. Calculations

A. Calculations are based upon the ratio of the peak area and concentration of standards to the peak area for the unknown. Peaks at the same or approximately the same retention times are compared. Once the method has been updated with the current calibration, this is calculated automatically by the software using linear regression. Remember that when dilutions are being run, the correct dilution factor must be entered.

B. Manual calculations are based upon the ratio of the peak and concentration of standards to the peak area for the unknown when the software will not automatically calculate the unknown concentration. Peaks at the same or approximately the same retention times are compared. The unknown concentration can be calculated from using this ratio. Remember that when dilutions are being run that the correct dilution factor must be entered before you will get the correct result.

C. When possible the unknown should be bracketed between two knowns and the calculation of the unknown made from both for comparison.

6. Quality Control

A quality control sample obtained from an outside source must first be used for the initial verification of the calibration standards. A fresh portion of this sample should be analyzed

every week to monitor stability. If the results are not within +/- 10 % of the true value listed for the control sample, prepare a new calibration standard and recalibrate the instrument. If this does not correct the problem, prepare a new standard and repeat the calibration. A quality control sample should be run at the beginning and end of each sample delivery group (SDG) or at the frequency of one per every ten samples. The QC's value should fall between ± 10 % of its theoretical concentration.

A duplicate should be run for each SDG or at the frequency of one per every twenty samples, whichever is greater. The RPD (Relative Percent Difference) should be less than 10%. If this difference is exceeded, the duplicate must be reanalyzed.

From each pair of duplicate analytes (X_1 and X_2), calculate their RPD value:

$$\% \ RPD = 2 \bullet \left(\frac{X_1 - X_2}{X_1 + X_2} \right) x \, 100$$

where:

(X_1 - X_2) means the absolute difference between X_1 and X_2.

7. Method Performance

The method detection limit (MDL) should be established by determining seven replicates that are 2 to 5 times the instrument detection limit. The MDL is defined as the minimum concentration that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte.

$$MDL = t_{(n-1,1-\alpha=99)}(S)$$

where:

t = the t statistic for n number of replicates used (for n=7, t=3.143)
n = number of replicates
S = standard deviation of replicates

8. Reference

EPA SW 846-9056, Chapter 5, September 1994

U.S. EPA Method 300.0, March 1984

ASTM vol. 11.01 (1996), D 4327, "Standard Test Method for Anions in Water by Chemically
Suppressed Ion Chromatography".

Total Kjeldahl Nitrogen Preparation

1. Discussion

Principle

Total Kjeldahl Nitrogen is the sum of organic nitrogen and ammonia nitrogen compounds of a sample. This method oxidizes all of the organic and inorganic nitrogenous compounds, at 100 to 110^{o}C, to nitrate. The digestion also helps dissolve solid material that could interfere with obtaining an accurate reading. The total nitrogen is then determined by the analysis of nitrate in the digestate with an IC. Total Kjeldahl Nitrogen is then determined by subtracting the pre-determined nitrite plus nitrate nitrogen values from the total nitrogen values.

Sensitivity

This method covers the range from 0.1 ppm to 2.9 ppm.

Interferences

Since this method is designed to oxidize ammonia to nitrate for analysis, the use of ammonia and/or ammonia based substances should be avoided in the work area and on the glassware, as this could produce increased positive results that are inaccurate.

Sample Preservation

This method cannot be performed on samples preserved in acid. Because of this, the samples should be prepped ASAP.

2. Safety

Wear a lab coat, gloves, and protective eyewear when prepping this experiment to avoid possible exposure to harmful substances.

3. Apparatus

CEM MARS Microwave Digestion Unit
Advanced Composite Vessels (ACV)
Graduated Cylinder
Wash Bottle
Automatic Pipettor

4. Reagents

Purity of Reagents—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society. Other grades may be used, provided it is first ascertained that the reagent is sufficiently high in purity to permit its use without lessening the accuracy of the determinations.

Purity of Water—Unless otherwise indicated, references to water shall be understood to mean Type I reagent grade water (Milli Q Water System) conforming to the requirements in ASTM Specification D1193.

Borate Buffer Solution—Dissolve 61.8 g H_3BO_3 and 8.0 g NaOH in a 1 L volumetric flask containing at least 500 mL of DI water. Swirl to mix and bring to volume. Make fresh every 3 months.

Digestion Reagent—Dissolve 20.1 g of $K_2S_2O_8$ and 3 g of NaOH in a 1 L flask containing at least 500 mL of DI water. Swirl to mix and bring to volume. Make fresh every 3 months.

Quality Control—Commercially available wastewater TKN standard (Environmental Resource Associates, "Ready-To-Use Wastewater QC Standards", Cat # 743, Arvada CO, 1-800-ERA-0122)

Glutamic Acid Stock Standard ($C_3H_5NH_2$ (COOH) $_2$), 100 ppm—Dry Glutamic Acid in oven at 105°C for 24 hours. Cover and place in dissector until cool. Dissolve 1.051g in DI water and dilute to 1 L; preserve with 2 mL chloroform ($CHCl_3$). Store in refrigerator for no longer than 6 months.

Nitrate Stock Standard (NO_3-N), 1000 ppm—Dry Potassium Nitrate (KNO_3) in oven at 105°C for 24 hours. Cover and place in dissector until cool. Dissolve 0.7218g in DI water and bring to 1 L; preserve with 2 mL chloroform ($CHCl_3$). Store in refrigerator for no longer than 6 months.

Nitrate Working Standard, 10 ppm—Dilute 100 mL of Nitrate Stock Standard to 1000 mL in 1 L flask. Preserve with 2 mL chloroform (CHCl3). Store in refrigerator for no longer that 6 months.

5. Procedure

A. Turn on the CEM MSP 1000 Microwave Digestion Unit and allow it to warm up for at least 15 minutes.

B. Standards Prep

1. *Using the 100 ppm Glutamic Acid Stock Standard, prepare the following*:
   a. 0.4 ppm = 1 mL of 100 ppm diluted to 250 mL
   b. 0.8 ppm = 2 mL of 100 ppm diluted to 250 mL
   c. 1.6 ppm = 4 mL of 100 ppm diluted to 250 mL
2. *Using the 10 ppm Nitric Stock Standard, prepare the following*:
   a. 0.1 ppm = 1 mL of 10 ppm diluted to 100 mL
   b. 0.2 ppm = 2 mL of 10 ppm diluted to 100 mL
   c. 0.4 ppm = 4 mL of 10 ppm diluted to 100 mL
   d. 0.8 ppm = 8 mL of 10 ppm diluted to 100 mL
   e. 1.6 ppm = 16 mL of 10 ppm diluted to 100 mL
   f. 2.9 ppm = 29 mL of 10 ppm diluted to 100 mL
3. *The QC is diluted from the ordered solution*: perform an appropriate dilution creating a QC with a value midway on calibration curve, (~1.5 ppm) using the ordered standard.
4. If it is deemed necessary, ICV's (Initial Calibration Verification) and CCV's (Continued Calibration Verification) can be run using a 0.8 ppm and/or 1.6 ppm glutamic acid solution.

C. Sample Prep

1. The Prep Blank is 10 mL of reagent grade DI water poured into the first liner.
2. For all samples and QC, a 10 mL aliquot should be poured into one of the advanced composite vessels, or ACV, liners.
3. Add 5 mL of Digestion Reagent to each liner.
4. Assemble the ACV system as described in **Microwave Digestion GLP**.

D. Digestion Set Up

1. From the options on the main menu of the microwave, press F3—"Recall Method/Data".
2. Press F1—"Recall Stored Method".
3. Use arrow keys to scroll down to "TKN SM"; press "Enter".
4. Press F1—"Load Program".
5. Press F4—"Start".
6. Press F1—"Yes". Once a digestion is started, watch the temperature probe and pressure tube carefully to make sure they do not become tangled up. If they do become tangled, press F1 to abort the run and remedy the problem.
7. Once the run is complete, disassemble the ACV's, add 1 mL of Borate Buffer Solution to each liner (all QC, samples, dups., etc.) and pour the digested samples into appropriately labeled precleaned containers.
8. The digested QC and samples, along with the corresponding data sheets, are to be transferred to the IC for analysis.

E. Prep-Batching

1. Log-on to the "Labworks" system.
2. Click on "Edit Data".
3. Enter the SDG number or choose it from the list.
4. Click on "OK".
5. Click on "OK".
6. In the row for TKN prep work (TKN_PREP), enter a 1 under the number of each sample completed and save it.
7. Exit system.

6. Quality Control

A duplicate sample should be prepped at the frequency of one per every twenty samples (sufficient sample permitting), or one per SDG, whichever is greater. The RPD should be less than 10%. If this difference is exceeded, the duplicate may need to be reprepped. The QC's value should fall between ± 10 % of its theoretical concentration as well.

7. References

Standard Methods for the Examination of Water and Wastewater, 20th edition (1998), Method 4500-N C, pg. 4-102